



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12Q 1/68, 1/48, C12N 15/63, 15/54, 9/12, C07H 15/12	A1	(11) International Publication Number: WO 94/12668 (43) International Publication Date: 9 June 1994 (09.06.94)
(21) International Application Number: PCT/US93/11569 (22) International Filing Date: 29 November 1993 (29.11.93) (30) Priority Data: 07/985,698 30 November 1992 (30.11.92) US (60) Parent Application or Grant (63) Related by Continuation US 07/985,698 (CIP) Filed on 30 November 1992 (30.11.92) (71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, Box OTT, Bethesda, MD 20892-9902 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): MOSS, Joel [US/US]; 8200 Wisconsin Avenue, Apartment 610, Bethesda, MD 20814 (US). OKAZAKI, Ian [US/US]; 5901 Montrose Road, #S-1205, Rockville, MD 20852 (US). ZOLKIEWSKA, Anna [US/US]; 5526 Besley Court, Apartment 205,		Rockville, MD 20851 (US). NIGHTINGALE, Maria, S. [US/US]; 7709 Mary Knoll Court, Bethesda, MD 20817 (US). (74) Agents: ALTMAN, Daniel, E. et al.; Knobbe, Martens, Olson & Bear, 620 Newport Center Drive, Suite 1600, Newport Beach, CA 92660 (US). (81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: MAMMALIAN MUSCLE NAD:ARGININE ADP-RIBOSYLTRANSFERASE		
(57) Abstract This invention relates to the identification and molecular characterization of NAD:arginine ADP-ribosyltransferases. Sequences from the rabbit skeletal muscle NAD:arginine ADP-ribosyltransferase and the human NAD:arginine ADP-ribosyltransferase are provided herein. Methods are disclosed for the isolation and identification of these gene sequences.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

MAMMALIAN MUSCLE NAD: ARGININE ADP-RIBOSYLTRANSFERASE**FIELD OF THE INVENTION**

5 This invention relates to vertebrate ADP-ribosyltransferases and specifically to mono-ADP-ribosyltransferases. In particular this invention relates to the purification, isolation and identification of mono-

10 NAD:arginine ADP-ribosyltransferases.

BACKGROUND OF THE INVENTION

10 Mono-ADP-ribosylation is a post-translational event resulting in the covalent modification of proteins. ADP-ribosyltransferases and ADP-ribosylarginine hydrolases are responsible for the forward and reverse reactions that control

15 the ADP-ribosylation of cellular proteins. In some systems, i.e. bacterial toxin ADP-ribosyltransferases, the extent of protein ADP-ribosylation is a critical determinant of enzymatic activity.

20 Mono-ADP-ribosylation is involved in the action of bacterial toxins and in the regulation of cellular processes in prokaryotes and eukaryotes (Moss, et al. *Adv. Enzymol.* 61: 303-379 (1988); Lowery, et al. and Williamson, et al. ADP-ribosylating Toxins and G Proteins: Insights into Signal Transduction, (1990) eds. Moss, J. et al. pp. 459-477 and pp.

25 493-510). Cholera toxin is a secretory product of the microorganism *Vibrio cholera*. This toxin is responsible for the pathogenesis of cholera. The cholera toxin directs the ADP-ribosylation of guanine nucleotide-binding proteins which in turn enhances their activity and increases the

30 responsiveness of some animal cells to various hormones, neurotransmitters and drugs (Moss, et al. supra and Ueda, K., et al. *Ann. Rev. Biochem.* 54:73-100, 1985).

35 Several ADP-ribosylating toxins have been cloned from bacteria (Nicosia, et al. *Proc. Natl. Acad. Sci. (USA)* 83: 4631-4635, 1986 and Nemoto, et al. *J. Biol. Chem.* 266: 19312-19319, 1991) and the crystal structures of some of the toxins have been solved (Allured, et al. *Proc. Natl. Acad. Sci. (USA)*

-2-

83: 1320-1324, 1986 and Sixma, et al. *Nature* 351: 371-377, 1991). While the bacterial toxins have similarities to one another in their amino acid sequences, the enzymes differ in the amino acids that they modify. Arginine, cysteine, asparagine and diphthamide (modified histidine) serve as ADP-ribose acceptors for Cholera toxin, pertussis toxin, botulinum C3 transferase and diphtheria toxin respectively.

Within prokaryotic and eukaryotic cells, ADP-ribosylation appears to be a reversible modification of proteins. An ADP-ribosylation cycle is involved in the regulation of the nitrogenase of the photosynthetic bacterium *Rhodospirillum rubrum* (Lowery, et al. supra). Here, an ADP-ribosyltransferase is responsible for the inactivation of the nitrogenase, whereas an ADP-ribosylarginine hydrolase releases the ADP-ribose moiety and activates the nitrogenase.

The role of mono-ADP-ribosylation in eukaryotes is less well-characterized; however, it is postulated that families of mono-ADP-ribosyltransferases will be identified in a given species and that these mono-ADP-ribosyltransferases will share homologies within their gene sequences. Eukaryotic mono-ADP-ribosyltransferases are believed to be involved in a number of physiological processes such as the regulation of adenyl cyclase (Obara, et al. *Eur. J. Biochem.* 200: 75-80, 1991; Brune, et al. *Proc. Natl. Acad. Sci. USA* 87: 3304-3308, 1990; Fendrick, et al. *Eur. J. Biochem.* 205: 25-31 (1992); and Kharadia, et al. *Exp. Cell. Res.* 201: 33-42, 1992). While it is believed that there are families of mono-ADP-ribosyltransferases located in different tissues, the gene sequences of this invention will be useful for verifying this hypothesis. Arginine- and cysteine-specific ADP-ribosyltransferases and ADP-ribosylarginine and ADP-ribosylcysteine hydrolases have been identified in animal tissues, consistent with the presence of ADP-ribosylation cycles responsible for the reversible ADP-ribosylation of arginine and cysteine residues in proteins (Moss, et al. *Proc. Natl. Acad. Sci. USA* 82: 5603-5607, 1985; Tanuma, et al. *J. Biol. Chem.* 263: 5485-5489, 1988; and Tanuma, et al. *FEBS*

Lett. 261: 381-384, 1990).

ADP-ribosylarginine hydrolase has been purified from turkey erythrocytes and rat brain. Further, the ADP-ribosylarginine hydrolase has been cloned from rat brain (Moss, et al. *J. Biol. Chem.* 267: 10481-10488, 1992). NAD: Arginine ADP-ribosyltransferases have been purified from turkey erythrocytes (Moss, et al. *J. Biol. Chem.* 255: 5838-5840, 1980; Yost, et al. *J. Biol. Chem.* 258: 4926-4929, 1983) and rabbit skeletal muscle (Taniguchi, et al. *Biochem. Biophys. Res. Commun.* 164: 128-133, 1989 and Peterson, et al. *J. Biol. Chem.* 265: 17062-17069, 1990). However, the gene sequences for these enzymes have remained unidentified until now. In turkey erythrocytes there is a family of ADP-ribosyltransferase enzymes that differ in their localization within the cell as well as in their physical, regulatory and kinetic properties (Williamson, et al., Moss, et al. *J. Biol. Chem.*, Yost, et al, all supra, and West, et al. *Biochemistry* 25: 8057-8062, 1986). The turkey ADP-ribosyltransferases appear to be ubiquitous in their tissue distribution, while the rabbit ADP-ribosyltransferase is located primarily within the sarcoplasmic reticulum of cardiac and skeletal muscle. Neither the RNA nor the DNA sequence of any mono-ADP-ribosyltransferases have been previously identified from a eukaryotic system

There are significant differences between the bacterial ADP-ribosyltransferases and eukaryotic ribosyltransferases. For example, since the bacterial toxins differ from the animal transferases in substrate specificity, therapies directed toward ADP-ribosyltransferases cannot rely on cloned bacterial enzymes. Eukaryotic ADP-ribosyltransferases are required for this work. In addition, the bacterial enzymes differ from their mammalian counterpart in their sensitivity to inhibitors. Therefore, it would be more valuable to test the effect of different inhibitors on eukaryotic enzymes than on their bacterial counterparts. Finally, bacterial transferases are targeted by a system different from those used with the animal transferases and therefore, the recombinant bacterial

enzymes may localize to different compartments within animal cells. The bacterial toxins function by binding to the outside of a eukaryotic cell and delivering their catalytic subunit to the cells. The eukaryotic enzymes are
5 intracellular enzymes that are required for effective protein regulation.

Few ADP-ribosyltransferases have been purified from animal cells. Moss, et al. purified an ADP-ribosyltransferase from turkey erythrocytes. In another example, Peterson, et
10 al. (supra), purified an enzyme from the same organ system and species used in the instant invention. This enzyme had an activity in vitro that was predictive of a mono-ADP-ribosyltransferase; however, the protein was not purified to a level that would permit someone to obtain useful tryptic
15 digest information. Prediction of the gene sequence requires tryptic digest information. While the protein of Peterson, et al. can be used to study the enzymatic properties of a mono-ADP-ribosyltransferase, gene therapeutic strategies cannot be pursued nor can studies be conducted to assess the effect of
20 the ADP-ribosyltransferase, or a mutated ADP-ribosyltransferase, on cell metabolism. Modification of cellular metabolism requires an ADP-ribosyltransferase gene, as produced in the present invention. Similarly, to develop a therapeutic modality in humans, a human enzyme is
25 particularly preferred primarily for immunological reasons.

Once a vertebrate ADP-ribosyltransferase is identified, the gene can be used to isolate other ADP-ribosyltransferases, including the human counterpart. The human sequence is heretofore undefined. Therefore, it is an object of the
30 present invention to identify the gene sequence for vertebrate mono-ADP-ribosyltransferases in general and for human mono-ADP-ribosyltransferase in particular.

SUMMARY OF THE INVENTION

This invention provides the amino acid and nucleotide sequence of a rabbit and human mono-ADP-ribosyltransferase. Oligonucleotide fragments from these sequences are useful for the further identification and isolation of homologous mono-ADP-ribosyltransferases isolated from other vertebrates. Nucleotide and peptide fragments derived from these sequences are useful for the development of assays to detect the presence of the enzyme in a tissue or fluid sample from a vertebrate.

In one embodiment of the present invention, Applicants disclose an assay method for identifying a mono-ADP-ribosyltransferase gene sequence from a vertebrate comprising (a) harvesting tissue containing ADP-ribosyltransferase activity from a vertebrate, (b) purifying the mono-ADP-ribosyltransferase from the tissue, (c) obtaining fragments of the mono-ADP-ribosyltransferase, (d) sequencing peptides obtained from the fragments, (e) preparing degenerate oligonucleotides corresponding to the amino acid sequence of the peptides, (f) using the oligonucleotides in at least one polymerase chain reaction to generate nucleic acid sequences, wherein the resulting fragments correspond to at least a portion of the mono-ADP-ribosyltransferase sequence, (g) generating the nucleic acid sequence of the polymerase chain reaction fragments, (h) identifying different oligonucleotides corresponding to the mono-ADP-ribosyltransferase sequence, and (i) repeating steps (f) through (h) until the complete nucleic acid sequence is identified.

In another embodiment of the present invention an assay method is disclosed for identifying a mono-ADP-ribosyltransferase gene sequence in a vertebrate comprising (a) identifying tissue from the vertebrate that contains ADP-ribosyltransferase activity, (b) isolating mRNA from the tissue, (c) preparing cDNA from the mRNA, (d) preparing an oligonucleotide pair suitable for use in a polymerase chain reaction, one oligonucleotide of the pair having a sequence substantially the same as a first portion of SEQ ID NO:1, and

-6-

the other oligonucleotide of the pair having a sequence substantially complementary to a second portion of SEQ ID NO: 1, (e) performing the polymerase chain reaction on the cDNA using the oligonucleotide pair of step (d) to generate PCR-amplified fragments, (f) sequencing the fragments generated from the polymerase chain reaction, and (g) repeating steps (e) through (g) until the cDNA is completely sequenced.

In another preferred embodiment of the present invention, an isolated or purified nucleic acid fragment encoding rabbit skeletal muscle ADP-ribosyltransferase is provided that corresponds to SEQ ID NO:1. Alternatively, the gene sequence is provided wherein the sequence is mutated in vitro to contain at least one nucleotide change in the sequence. Purified or isolated oligonucleotide is also provided that comprises at least 15 continuous bases of SEQ ID NO:1. In addition recombinant gene vectors are provided that contain at least a 15 base portion of SEQ ID NO:1 as well as recombinant protein expressed from the recombinant gene vector. Preferably, this protein is essentially pure and the protein exhibits ADP-ribosyltransferase activity. The recombinant protein is preferably expressed in eukaryotes or prokaryotes. In another preferred embodiment, gene sequences are disclosed that have at least an 85% homology to SEQ ID NO:1.

In another preferred embodiment a gene sequence is provided that encodes human ADP-ribosyltransferase, consisting essentially of the sequence corresponding to SEQ ID NO:37. In addition, recombinant gene vectors are provided that contain at least 15 continuous bases of SEQ ID NO:37. Recombinant protein expressed from the recombinant gene vector are disclosed. Preferably, the recombinant protein is essentially pure. More preferably, the recombinant protein exhibits ADP-ribosyltransferase activity. The recombinant protein is expressed in eukaryotes or prokaryotes.

In yet another preferred embodiment purified antibody is provided that is capable of specifically binding to the recombinant protein encoded by SEQ ID NO:1. In another embodiment, purified antibody is provided that is capable of

-7-

specifically binding to the recombinant protein encoded by SEQ ID NO:37.

In another aspect of the present invention, there is provided an assay method for detecting an ADP-ribosyltransferase gene sequence homologous to SEQ ID NO:37 in a vertebrate comprising (a) obtaining at least one oligonucleotide pair from SEQ ID NO:37 suitable for a polymerase chain reaction and a third oligonucleotide pair selected from SEQ ID NO:3 positioned between the oligonucleotide pair, (b) isolating a tissue sample from a vertebrate, (c) processing the tissue to obtain nucleic acid suitable as a template for use in a polymerase chain reaction, (d) performing a polymerase chain reaction using the oligonucleotide pair to generate at least one DNA fragment, (e) hybridizing the third oligonucleotide with the DNA fragment and (f) detecting hybridization between the third oligonucleotide and the DNA fragment.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 is a hydrophilicity plot of the predicted amino acid sequence of the rabbit ADP-ribosyltransferase.

DETAILED DESCRIPTION OF THE INVENTION

The gene sequence for rabbit and human muscle NAD: Arginine ADP-ribosyltransferase is disclosed. In addition, methods are disclosed for isolating and identifying sequences corresponding to NAD: Arginine ADP-ribosyltransferase from other vertebrates.

Knowledge of the gene sequence is required in order to study the effects of the enzyme on cells both in vivo and in vitro. Recombinant NAD: Arginine ADP-ribosyltransferase nucleic acid can be introduced into cells to alter the level of protein ADP-ribosylation and to modify intracellular protein activity in general. The ADP-ribosyltransferase gene, when overexpressed, can also be used to study the effect of pharmacological agents on endogenous ADP-ribosylation. Further, the identification of the gene sequence and the expression of this sequence in appropriate eukaryotic or

prokaryotic cells permits the isolation of this protein in amounts suitable for purification for antibody production, the development of diagnostic reagents, and sensitive tests to detect the activity of this enzyme in cell lysates. Nucleic acid fragments of this sequence are useful as genetic probes for assessing differences in ADP-ribosyltransferase expression within a population and for the identification of ADP-ribosyltransferase mutants. The isolation of purified recombinant protein facilitates production of tests to identify inhibitors and activators of the ADP-ribosyltransferase. These agents would likely have therapeutic value in the medical community.

To identify the gene sequence, the enzyme is first purified from mammalian muscle. In Example 1, the source of enzyme was rabbit skeletal muscle. It is contemplated that the procedures disclosed herein are suitable for a variety of muscle tissue from a variety of vertebrates. While Example 1 provides a specific exemplary method, there are also a number of methods recognized in the art to purify active enzyme from tissue homogenates. The purification scheme selected should yield suitable quantities of enzyme (at least 100 picomoles) at a suitable level of purity (at least 80% pure).

The initial purification steps used in this invention (through concanavalin A agarose) were those described by Peterson, et al. (supra), with several important modifications (Table 1 and Example 1). The specific activity of the transferase identified by Peterson, et al., used two chromatographic steps (DE52 cellulose and concanavalin A agarose) to generate enzymatic activity ranging from 0.13 to 5.1 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ measured with 2 mM NAD and 10 mM L-arginine methyl ester. The assay to measure the specific activity of the enzyme is described by Larew, et al. (*J. Biol. Chem.* 266: 52-57 1991). Analysis of the enzyme fraction purified on DE52 cellulose (Whatman, Maidstone, England and concanavalin A agarose, revealed a significant level of impurity as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This level of impurity was too

-9-

high to permit effective amino acid sequencing as evidenced by contaminating protein of equal molecular weight. Therefore, two additional purification steps were introduced; high resolution DEAE chromatography and gel filtration-high pressure liquid chromatography (HPLC). This combination dramatically improved the purity of the transferase, as described in Example 1. The final purity was assessed by Gel filtration-HPLC (not shown). Tryptic digestion and amino acid sequencing of tryptic peptides were performed following nondenaturing gel filtration HPLC.

TABLE 1. PURIFICATION SUMMARY OF ADP-RIBOSYLTRANSFERASE
FROM RABBIT SKELETAL MUSCLE

	Purification step	Protein	Units	Specific activity
	Purification	Yield		
	(-fold)	(%)	(mg)	(mmol/min)
				(mmol . min ⁻¹ . mg ⁻¹)
20	15,000g supernatant	26,000	1.70	0.000065
	1	100		
	KCl-washed pellet	1,400	1.20	0.00086
	13	71		
	DE52	120	0.82	0.0068
25	105	48		
	Concanavalin A agarose	4	0.57	0.14
	2,150	34		
	DEAE MemSep	0.067	0.29	4.3
	66,150	17		
30	Gel filtration HPLC	0.030	0.27	9.0
	138,500	16		
	Gel filtration HPLC (+1% SDS)	0.005	0.07	14
	215,400	4		

While there are a variety of purification schemes that can be used to obtain the purified enzyme corresponding to the amino acid sequence of this invention, those with skill in the art will recognize that the purification scheme should maximize protein yield and maintain protein integrity thereby maximizing enzymatic activity. Following the methods disclosed in Example 1, the ADP-ribosyltransferase was purified about 215,000-fold with respect to the 15,000g supernatant and at least 16,000-fold with respect to the

-10-

membrane fraction. The overall yield, which will vary according to the methods selected, was about 4% of the starting material using the purification strategy of Example 1 (see Table 1). The specific activity of the enzyme preparation was $14 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ when assayed with 0.1 mM NAD and 20 mM agmatine, and $68 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ with 2 mM NAD. The transferase apparently represented approximately 90% of the purified protein.

There was a significant discrepancy between the molecular size of the transferase, estimated from the mobility of the enzyme on gel filtration-HPLC (61 kDa), and the estimated molecular size predicted from SDS-PAGE (38 kDa). This discrepancy is consistent with the interaction of the protein with CHAPS, a zwitterionic detergent (Calbiochem, La Jolla, California) or alternatively with protein dimerization.

The purified enzyme preparation was subjected to tryptic digestion as described in Example 2. After running SDS-PAGE, proteins were electroblotted onto a nitrocellulose membrane and a band, corresponding to the ADP-ribosyltransferases, was excised and sent to Dr. William Lane (Harvard Microchem, Boston, MA). In situ tryptic digestions were performed as described (Aebersold, et al. *Proc. Natl. Acad. Sci. USA* 84: 6970-6974 (1987)). Trypsin was incubated with the piece of nitrocellulose (enzyme to substrate ratio of about 1:20). Cleaved peptides, released from the membrane, were separated by reverse-phase HPLC. Peptide-containing fractions were collected. Seven peptides, which had the highest absorption at 215 nm, were derivatized with phenylthiohydantoin and amino acid sequence analysis was performed in a gas-phase sequenator. The amino acid sequences of several tryptic peptides was determined and these are provided in Table 3 as underlined sequences. The amino acid sequence of one of the tryptic peptides of the purified ADP-ribosyltransferase (amino acids 74-87) was used to synthesize two sets of degenerate oligonucleotides, which were used as nested primers in PCR amplifications from a rabbit skeletal muscle cDNA library.

-11-

Cloning of an ADP-ribosyltransferase cDNA

As noted above, the sequence of a tryptic peptide corresponding to amino acids 74-87 in Table 3, was used to synthesize degenerate antisense oligonucleotides. Other primers, corresponding to the other tryptic peptides, could similarly be used in nested PCR reactions to identify the sequence of interest. The oligonucleotides B2, B3 and B4 (SEQ ID NOS:14-16, see also Table 2) were used in two sequential polymerase chain reactions (PCR) with oligonucleotides derived from the pBluescript plasmid sequence (BSC1, SEQ ID NO:17 and BSC2, SEQ ID NO:18, see Table 2) to identify candidate sequences from a Lambda ZAPII rabbit skeletal muscle library (see Example 2). Although both orientations of the primers were used, significant amounts of PCR product were obtained with the antisense primers (B2, B3 and B4, SEQ ID NOS: 14-16), based on amino acids 74-82, and sense plasmid primers BSC1 and BSC2, SEQ ID NOS:17 and 18, respectively. PCR fragments corresponded to the 5'-coding and untranslated region of the clone (positions -91 to 239 in Table 3). This PCR fragment was cloned into a suitable cloning vector (see Example 3) using methods well known in the art and subjected to dideoxynucleotide sequencing. Those with skill in the art will recognize that any number of commercially available cloning vectors could similarly be used to facilitate DNA sequencing. The deduced amino acid sequence of the cloned DNA fragment included a sequence that corresponded to one of the tryptic peptides (amino acids 31-58, see Table 3 and SEQ ID NO:2), thus confirming the identity of the clone.

-12-

TABLE 2

Amplification Primers

	<u>Name</u>	<u>Seq. ID No.</u>	<u>Description</u>
5	B2	14	Inverse complement of nucleotides encoding amino acids 74-80
	B3	15	Inverse complement of nucleotides encoding amino acids 76-82
	B4	16	Inverse complement of nucleotides encoding amino acids 76-82
	BSC1	17	Specific to pBluescript sequence
	BSC2	18	Specific to pBluescript sequence, 3' to BSC1
10	TG	19	Inverse complement of nucleotides encoding amino acids 52-58
	CAU-AC	20	Inverse complement of nucleotides encoding amino acids 45-51 (underlined) and a subcloning sequence at 5'-end
	R ₀ R _I T	21	(dT) 17 adaptor primer for 5'-RACE
	R ₀	22	Outer adaptor primer for 5'-RACE
	CUA-R _I	23	Inner adaptor primer for 5'-RACE (underlined) and subcloning sequence at 5'-end
15	5Ndel	24	Corresponding to amino acids 24-30 (underlined), a Ndel site (<i>italics</i>) plus subcloning sequence at 5'-end
	3BamHI	25	Inverse complement of nucleotides encoding amino acids 297-303 (underlined), a stop codon (double underlined), a BamHI site (<i>italics</i>), and a subcloning sequence at 5'-end
	5PRM	26	Inverse complement of nucleotides (-90)-(-43)
	48SP	27	Inverse complement of nucleotides encoding amino acids 31-46
	3PRM	28	Inverse complement of nucleotides 960-1007
20	HSM-5	29	Inverse complement of nucleotides 283-306
	HSM-CAUN	30	Inverse complement of nucleotides 250-268 and contains a subcloning sequence at 5'-end
	HSM-30	31	Inverse complement of nucleotides 212-242

-13-

	HSM-1F	32	Corresponding to nucleotides 857-876
	CAUHSM-2F	33	Corresponding to nucleotides 881-901 and contains a subcloning sequence at 5'-end
	P-RT	34	Inverse complement of nucleotides 1099-1122
	HSM-1N	35	Corresponding to nucleotides (-79)-(-49) containing a subcloning sequence at 5'-end
5	HSM-RN	36	Inverse complement of nucleotides 1057-1074 containing a subcloning sequence at 5'-end

Table 2 provides the sequences for the oligonucleotides that were used to identify the full length rabbit nucleic acid sequence of this invention. The letter "N" denotes any nucleotide A, C, G or T. Oligonucleotides are listed from their 5' to 3' end.

Based on the partial cDNA sequence, an oligonucleotide (48SP, SEQ ID NO:27, see Table 2) was synthesized for use as a probe to screen a skeletal muscle cDNA library. An exemplary screening strategy is provided in Example 4. Several clones were obtained, one of which contained a sequence that overlapped with the PCR product described above. This sequence extended from position -14 to 1020 (Table 3 and SEQ ID NO:1) and contained a 981-bp open reading frame, encoding a 36,134-kDa protein. The deduced amino acid sequence (SEQ ID NO:2, GenBank accession no. M98764) of this protein includes all seven amino acid sequences identified by tryptic digest from the purified transferase. Numbering is relative to the initiating methionine codon. Sequences identified by tryptic digest are underlined. Stop codons are double underlined. Asterisks identify potential N-glycosylation sites.

-14-

TABLE 3

Rabbit Skeletal Muscle NAD;
arginine ADP - ribosyltransferase

```

-105          GACCA TCACATGAAG CCAACACCAG CTCCCCTGCC CCGGACAAGG
-60  CCTAGATGAG GAAAGTAAGA GTCAAAAGGA GAGAGAACT GGCCTGGGGT GGCCCCAACC
      1  ATGTGGGTTC CTGCCGTGGC GAATCTGCTC CTTCTGTCCC TGGGCCTTCT GGAAGCAATT
      1  M W V P A V A N L L L L S L G L L E A I
      61  CAGGCCCAGA GCCACCTGGT CACACGTCGA GACCTCTTCT CTCAAGAGAC ACCGCTGGAC
      21  Q A Q S H L V T R R D L F S O E T P L D
     121  ATGGCCCCGG CCTCCTTTGA TGACCAGTAC GTCGGCTGTG CAGCAGCCAT GACAGCTGCC
      41  M A P A S F D D O Y V G C A A A M T A A
     181  CTCCCGCATC TCAACCTCAC GGAGTTCCAG GTCAACAAAG TGTATGCGGA CGGCTGGGCA
      61  L P H L N L T E F Q V N K V Y A D G W A
     241  CTGGCAAGCA GCCAGTGGCG GGAGCGCTCG GCCTGGGGGC CCGAGTGGGG CCTCAGCACA
      81  L A S S Q W R E R S A W G P E W G L S T
     301  ACCCGGCTCC CCCC GCCGCC TGC GGGATT T CGGGATGAAC ACGGGGTGGC CCTGCTGGCC
     101  T R L P P P P A G F R D E H G V A L L A
     361  TACACGGCCA ACAGCCCCCT ACACAAGGAG TTCAATGCCG CGGTACGCCA GCGGGGCCGC
     121  Y T A N S P L H K E F N A A V R Q A G R
     421  TCCCGAGCCC ACTACCTCCA GCACTTCTCC TTCAAGACCC TGCATTCTCT GCTGACCGAG
     141  S R A H Y L Q H F S F K T L H F L L T E
     481  GCCCTGCAGC TGCTGGGCAG GGATCAGCGA ATGCCAGAT GCCGTCAGGT GTTCCGGGGG
     161  A L Q L L G R D Q R M P R C R Q V F R G
     541  GTGCATGGAC TGCGCTTCCG GCCAGCAGGG CCCGGGACCA CTGT CAGGCT GGGGGGCTTT
     181  V H G L R F R P A G P G T T V R L G G F
     601  GCCTCTGCGT CACTGAAAAA TG TAGCAGCC CAGCAGTTTG GCGAGGACAC GTTCTTTGGC
     201  A S A S L K N V A A Q Q F G E D T F F G
     661  ATCTGGACCT GCCTTGGGGT CCCTATCCAG GGCTACTCCT TTTTCCCTGG GGAGGAGGAG
     221  I W T C L G V P I Q G Y S F F P G E E E
     721  GTTCTGATCC CCCCCTTTGA GACCTTCCAG GTCATCAACG CCAGCAGACC TGCCAGGGC
     241  V L I P P F E T F Q V I N A S R P A Q G
     781  CCTGCCCCGA TCTACCTGAA GGCGCTGGGC AAGCGCAGCT CATACTACTG CGAGTACATC
     261  P A R I Y L K A L G K R S S Y N C E Y I
     841  AAAGAAATGC AGTGCAAGTC TAGGCCCTGC CACCTGGACA ATTCAGCCTC GGCTCAGGAG
     281  K E M Q C K S R P C H L D N S A S A Q E

```

-15-

901 CGCCTCTCCA CAGCCTGGTC CCTCCTGCTG CTGCTCGCGT TCCTTGCGGT GGGGCCCTTC
301 R L S T A W S L L L L L A F L A V G P F

961 CCAGGAAGCC CAGGCCTCTT CTGACCCCCC AGACTCTGGA CATTCCTGCC TGCTGCCTCT
321 P G S P G L F End

-16-

The sequence was also obtained from poly (A)⁺ RNA isolated from rabbit skeletal muscle. A preferred exemplary procedure for obtaining the sequence from poly (A)⁺ RNA is provided in Example 5. The sequence of the ribosyltransferase was obtained by hybridizing primer TG, SEQ ID NO: 19 (Table 2) to the RNA to generate cDNA using an avian myeloblastosis virus (AMV) reverse transcriptase (Invitrogen, San Diego, California) under conditions described by Frohman, et al. (*Proc. Natl. Acad. Sci. USA* 85: 8998-9002, 1988. A 3' tail was added to the product using terminal deoxynucleotidyl transferase and the second strand was prepared by annealing primer R₀R₁T, SEQ ID NO:21, to the RNA and extending the primer with *Taq* DNA polymerase. Further amplification of the fragments, by PCR, was performed using primers TG, SEQ ID NO:19, and CAU-AC, SEQ ID NO:20, and primer pair R₀, SEQ ID NO:22, and CUA-R₁, SEQ ID NO:23. The final product was cloned into a suitable cloning vector such as pAMPI (CloneAmp subcloning system, GIBCO-BRL, Gaithersburg, Maryland) and sequenced. The sequence corresponded to positions -105 to 152 of Table 3 and was generated from 5'RACE techniques as outlined in Example 5.

Since the amino acid sequence of the N terminus of the ADP-ribosyltransferase was not identified using the techniques described above, supplemental techniques can be used to identify the position 1 methionine (Table 3 and SEQ ID NO:1). Northern analysis is used in Example 6 as one example of a method to determine the initiating methionine. The sequence of the PCR product obtained with degenerate primers B2, B3 and B4, SEQ ID NOS:14-16 (position -91 to 239) and the sequence of the 5'-RACE product (position -105 to 152) contained two in-frame stop codons upstream from the methionine codon at positions -54 to -52 and -45 to -43. Northern blot analysis indicated that oligonucleotide probes 5PRM, SEQ ID NO:26 (specific to the 5'-untranslated region, containing the two putative stop codons), 48SP, SEQ ID NO:27 (specific to the coding region) and 3PRM, SEQ ID NO:28 (specific to the 3'-end

-17-

of the coding region) hybridized to RNA of the same size (about 4 kb), consistent with the conclusion that the 5'-untranslated region is present in transferase mRNA.

5 Once the enzyme has been cloned and sequenced it is possible to use specific probes identified from the cloned sequence, or degenerate probes with substantial homology to the cloned sequence, to assess the tissue distribution of the ADP-ribosyltransferase in other tissues (see Example 6). An ADP-ribosyltransferase specific probe was hybridized to RNA
10 isolated from a variety of rabbit tissues. The probe recognized a 4-kb mRNA expressed primarily in skeletal and cardiac muscle tissues. The northern blots assessed the tissue distribution of the rabbit ribosyltransferase.

It is contemplated that a similar analysis could be
15 performed on tissues derived from other vertebrates using probes derived from the rabbit ADP-ribosyltransferase sequence. Similarly, degenerate probes corresponding to the rabbit ADP-ribosyltransferase sequence, hybridization at lower temperature, washes at reduced stringencies, or the like can
20 be used to identify ribosyltransferases from tissues of other vertebrates.

In mammals, cell lysates and partially purified protein preparations from cells indicate that arginine-specific ADP-ribosyltransferase enzymatic activity is predominantly found
25 in skeletal muscle and cardiac tissues (Soman, et al. *Biochem. Biophys. Res. Commun.* 120: 973-980, 1984). Recently, activity was also found in murine T-cell hybridoma, thymoma and lymphoma cells (Soman, et al. *Biochem. Biophys. Res. Commun.* 176: 301-308, 1991).

30 The sequence and amino acid data from the ADP-ribosyltransferase facilitates an analysis of the hydrophilicity and hydrophobicity of the enzyme. This analysis helps to identify functional regions of the protein and is necessary for structurally analyzing the catalytic core
35 of the enzyme. The hydrophilicity plot of the ribosyltransferase (Figure 1) indicates that the enzyme has

-18-

strongly hydrophobic amino and carboxyl termini and a hydrophilic center. These characteristics permit one with skill in the art to compare the functional regions of the protein with other enzymes known in the art. Here, the hydrophobic and hydrophilic pattern is common to glycoposphatidylinositol (GPI)-anchored membrane proteins (Ferguson, M.A.J. *Biochem. Soc. Trans.* 20: 243-256, 1992 and Udenfriend, et al. *Cell. Mol. Biol.* 38: 11-16, 1992). Hydrophilicity values were obtained with the MacVector program (IBI, a division of Kodak, New Haven, Connecticut) using the Kyte-Doolittle algorithm (provided in the MacVector program) using a window setting of 16 amino acids.

The hydrophobic N-terminal portion serves as a leader sequence, directing the enzyme into the endoplasmic reticulum. The hydrophobic sequence at the C terminus is recognized inside the ER as a signal for glycoposphatidylinositol modification.

Two potential sites for N-linked glycosylation were found in the deduced amino acid sequence of the transferase. These are Asp⁶⁵ and Asp²⁵³. Since the protein binds to a lectin column (concanavalin A agarose) and because phosphatidylinositol-linked proteins are often heavily glycosylated, it is likely that the ADP-ribosyltransferase is subject to these posttranslational modifications.

To conclusively show that the cloned enzyme is an arginine-specific mono-ADP-ribosyltransferase, the sequence was cloned into a suitable expression vector and expressed in either bacteria or eukaryotes. Examples 7 and 8 outline strategies for the expression of the ADP-ribosyltransferase in *E. coli* and eukaryotic cells, respectively. Since eukaryotic cells carry endogenous levels of ADP-ribosyltransferase, the levels of enzymatic activity identified in transfected eukaryotic cells should be compared with non-transfected or mock-transfected cells.

Expression of the full length ADP-ribosyltransferase in *E. coli* was attempted using constructs of the ADP-

-19-

ribosyltransferase either as a fusion protein of glutathione S-transferase or as a non-fusion protein. The protein was inactive using both constructs. ADP-ribosyltransferase activity was obtained in transformed *E. coli* using a construct that included amino acids 24-303 of the ADP-ribosyltransferase ligated as a non-fusion protein in pET3a (Novagen, Madison, Wisconsin). The truncated form of the protein lacked both the hydrophobic amino and carboxyl termini. In assays to assess the enzymatic activity of the protein, a product was formed that comigrated on an anion exchange HPLC column with the product (ADP-ribosylagmatine) formed by native rabbit skeletal muscle ADP-ribosyltransferase in the presence of NAD and agmatine.

In transformed rat mammary adenocarcinoma (NMU) cells transformed with the rabbit skeletal muscle ADP-ribosyltransferase cDNA sequence of SEQ ID NO: 1, significant ADP-ribosyltransferase activity was observed, with 62% occurring in the membrane fraction. ADP-ribosyltransferase activity was negligible in control NMU cells and cells transformed with either the vector alone or with the vector containing an antisense insert (Example 8).

Comparison of the Deduced Amino Acid Sequence of ADP-ribosyltransferase with Other Protein Sequences

A homology search of the deduced amino acid sequence of the transferase was done at the National Center for Biotechnology Information Bethesda, Maryland using the BLAST network service. The highest homology score was obtained for rat and mouse RT6.2 protein. This protein is expressed exclusively on postthymic T cells (Koch, et al. *Proc. Natl. Acad. Sci. USA* 87: 964-967, 1990). The regions of greatest similarity were amino acids 39-88 (42% identity), 214-254 (46% identity), 107-124 (72% identity), 148-166 (52% identity) and 194-206 (61% identity). RT6.2 is a 26-kDa phosphatidylinositol-linked protein, with hydrophobic amino and carboxyl termini. The predicted amino acid sequence of

-20-

the RT6.2 protein begins with a leader of 20 hydrophobic amino acids and ends with a hydrophobic stretch of 29 residues.

No significant homology was found between rabbit skeletal muscle transferase and various bacterial ADP-ribosylating toxins, the ADP-ribosyltransferase from *Rhodospirillum rubrum* or poly (ADP-ribose) polymerase. Thus, the skeletal muscle transferase is an unique enzyme, distinct from the bacterial transferases in structure and perhaps in substrate specificity.

Use of the Mammalian Sequence to Obtain Human mono-ADP ribosyltransferase

Based on the rabbit sequence provided in Table 3 and SEQ ID NO:1, two sets of nested degenerate primers were designed for use in two consecutive PCR amplifications to obtain the human ADP-ribosyltransferase sequence from isolated human skeletal muscle poly (A)⁺ RNA. Although a preferred method for isolating SEQ ID NO: 3 is provided in Example 10, other primer pairs for both 5'-RACE and 3'-RACE are contemplated including 5' GCTGTCTGCATACACCTGGTTGGC 3' (SEQ ID NO: 10; inverse complement of bases 80-103 in the human fragment) and 5' GTGGTTGAGATCCGGGAGAGC 3' (SEQ ID NO: 11; inverse complement of bases 47-67 in the human fragment) for 5'-RACE and 5' CCCGCATCTACCTCCGAGCC 3' (SEQ ID NO: 12; bases 54-73 in the human fragment) and 5' CAAGCACAGCACCTATAATT 3' (SEQ ID NO: 13; bases 679-698 in the human fragment). A partial cDNA sequence, encoding a 224 amino acid fragment of human skeletal muscle mono-ADP-ribosyltransferase, was obtained by PCR using primers based on the rabbit mono-ADP-ribosyltransferase sequence.

Primers for the first PCR reaction:

1A:	(ACGT) TT (AG) GA (TC) ATGGC (ACGT) CC (ACGT) GC	SEQ ID NO:5
1B:	(ACGT) CT (ACGT) GA (TC) ATGGC (ACGT) CC (ACGT) GC	SEQ ID NO:6
2:	(TC) TT (AG) CA (TC) TGCAT (TC) TC (TC) TT	SEQ ID NO:7

Primers for the second PCR reaction:

3:	(AGCT) TT (TC) GA (TC) GA (TC) CA (AG) TA (TC) GT	SEQ ID NO:8
----	---	-------------

-21-

4: (AGT)AT(AG)TA(TC)TC(AG)CA(AG)TT(AG)TA SEQ ID NO:9

5 The bases in parentheses represent degenerate positions. Primers 1A and 1B correspond to amino acids 38-44 in the rabbit ADP-ribosyltransferase sequence and primer 2 is an inverse complement of nucleotides encoding amino acids 281-286. Primer 3 is internal to 1A and 1B and its sequence corresponds to amino acids 45-51. Primer 4 is internal to primer 2 and is an inverse complement of nucleotides encoding
10 amino acids 275-280.

The resulting PCR product (about 670 base pairs) was subcloned into a vector (pAmp1, GIBCO-BRL) and sequenced by the dideoxy sequencing methods previously described. Rapid amplification of cDNA ends (RACE) was used to determine
15 sequentially the 5' and 3' ends of the human transferase mRNA as described in Example 11. The full length human sequence (SEQ ID NO: 37) is shown in Table 4. The deduced amino acid sequence of the 224 amino acid fragment of SEQ ID NO:37, as determined from the nucleotide sequence, was 87% identical to
20 that of the rabbit mono-ADP-ribosyltransferase (see Table 5, SEQ ID NO:4).

Completion of the Human ADP-ribosyltransferase sequence

25 The human ADP-ribosyltransferase sequence provided in Table 4, SEQ ID NO:3 and SEQ ID NO:4 is a partial sequence. Both the amino and carboxyl ends remain unidentified. The remaining sequence of the gene was obtained using 5'-RACE and 3'-RACE methods. These techniques are disclosed in the art and permit the rapid amplification of the 5' end and the 3'
30 end of the cDNA. For a detailed protocol see Frohman, et al. *Technique- A Journal of Methods in Cell and Molecular Biology* 1: 165-170 1989). The 5'-RACE methodology is described in Example 5 and both the 5'-RACE and 3'-RACE methods are detailed in Example 11.

-22-

TABLE 4
NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCE OF HUMAN ADP-RIBOSYLTRANSFERASE

TTCCACCAGG ACAGGCCTAG ATGAGGAAAC TGAGACCCAA AAAGAGACAG CAACTGGCCC	60
AGGGTCACCA GC ATG CAG ATG CCT GCT ATG ATG TCT CTG CTT CTT GTG	108
Met Gln Met Pro Ala Met Met Ser Leu Leu Leu Val	
1 5 10	
TCT GTG GGC CTC ATG GAA GCA CTT CAG GCC CAG AGC CAC CCC ATC ACA	156
Ser Val Gly Leu Met Glu Ala Leu Gln Ala Gln Ser His Pro Ile Thr	
15 20 25	
CGA CGA GAC CTC TTC TCT CAA GAG ATT CAG CTG GAC ATG GCC CTG GCC	204
Arg Arg Asp Leu Phe Ser Gln Glu Ile Gln Leu Asp Met Ala Leu Ala	
30 35 40	
TCC TTT GAT GAC CAG TAC GCT GGC TGT GCT GCT GCC ATG ACA GCT GCT	252
Ser Phe Asp Asp Gln Tyr Ala Gly Cys Ala Ala Ala Met Thr Ala Ala	
45 50 55 60	
CTC CCG GAT CTC AAC CAC ACG GAG TTC CAG GCC AAC CAG GTG TAT GCA	300
Leu Pro Asp Leu Asn His Thr Glu Phe Gln Ala Asn Gln Val Tyr Ala	
65 70 75	
GAC AGC TGG ACA CTG GCA AGC AGC CAA TGG CAG GAG CGT CAG GCC AGG	348
Asp Ser Trp Thr Leu Ala Ser Ser Gln Trp Gln Glu Arg Gln Ala Arg	
80 85 90	
TGG CCA GAG TGG AGT CTC AGC CCC ACC CGT CCA TCC CCG CCA CCC CTG	396
Trp Pro Glu Trp Ser Leu Ser Pro Thr Arg Pro Ser Pro Pro Pro Leu	
95 100 105	
GGC TTC CGC GAT GAG CAT GGG GTG GCC CTC CTG GCC TAC ACA GCC AAC	444
Gly Phe Arg Asp Glu His Gly Val Ala Leu Leu Ala Tyr Thr Ala Asn	
110 115 120	
AGC CCC CTG CAC AAG GAG TTC AAT GCA GCC GTG CGT GAG GCG GGC CGC	492
Ser Pro Leu His Lys Glu Phe Asn Ala Ala Val Arg Glu Ala Gly Arg	
125 130 135 140	
TCC CGG GCC CAC TAC CTC CAC CAC TTC TCC TTC AAG ACA CTC CAT TTC	540
Ser Arg Ala His Tyr Leu His His Phe Ser Phe Lys Thr Leu His Phe	
145 150 155	
CTG CTG ACT GAG GCC CTG CAG CTC CTG GGC AGC GGC CAG CGT CCA CCC	588
Leu Leu Thr Glu Ala Leu Gln Leu Leu Gly Ser Gly Gln Arg Pro Pro	
160 165 170	
CGG TGC CAC CAG GTG TTC CGA GGT GTG CAC GGC CTG CGC TTC CGG CCA	636
Arg Cys His Gln Val Phe Arg Gly Val His Gly Leu Arg Phe Arg Pro	
175 180 185	

-23-

GCA GGG CCC CGG GCC ACC GTG AGG CTG GGG GGC TTT GCT TCT GCC TCC Ala Gly Pro Arg Ala Thr Val Arg Leu Gly Gly Phe Ala Ser Ala Ser 190 195 200	684
CTG AAG CAT GTT GCA GCC CAG CAG TTT GGT GAG GAC ACC TTC TTC GGC Leu Lys His Val Ala Ala Gln Gln Phe Gly Glu Asp Thr Phe Phe Gly 205 210 215 220	732
ATC TGG ACC TGC CTT GGG GCC CCT ATC AAG GGC TAC TCC TTC TTC CCT Ile Trp1Thr Cys Leu Gly Ala Pro Ile Lys Gly Tyr Ser Phe Phe Pro 225 230 235	780
GGA GAG GAA GAG GTG CTG ATC CCC CCC TTT GAG ACC TTC CAA GTG ATC Gly Glu Glu Glu Val Leu Ile Pro Pro Phe Glu Thr Phe Gln Val Ile 15 240 245 250	828
AAT GCC AGC AGA CCG GCC CAG GGC CCC GCC CGC ATC TAC CTC CGA GCC Asn Ala Ser Arg Pro Ala Gln Gly Pro Ala Arg Ile Tyr Leu Arg Ala 255 260 265 20	876
CTG GGC AAG CAC AGC ACC TAC AAC TGC GAG TAC ATC AAA GAC AAG AAG Leu Gly Lys His Ser Thr Tyr Asn Cys Glu Tyr Ile Lys Asp Lys Lys 270 275 280	924
TGC AAG2TCT GGG CCT TGC CAT CTG GAT AAT TCA GCC ATG GGT CAG AGC Cys Lys Ser Gly Pro Cys His Leu Asp Asn Ser Ala Met Gly Gln Ser 285 290 295 300	972
CCC CTC TCT GCA GTC TGG TCT TTG CTG CTG CTG CTC TGG TTC CTC GTG Pro Leu3Ser Ala Val Trp Ser Leu Leu Leu Leu Leu Trp Phe Leu Val 305 310 315	1020
GTG AGG GCC TTT CCA GAT GGT CCA GGC CTC CTT TGATGCATGA GACA Val Arg Ala Phe Pro Asp Gly Pro Gly Leu Leu 35 320 325	1067
CGGGAC AGCCTCGCCTGCTG CCTCTGCCCA TCCTGAGGAT GTTGGCCATG TGTGCTTCAG	1127
TGTAACCAAG ATTCCTGTCA ATCCCATCTG CAGGGAACTC TGGGACCTTC TCTGGTAGCT 40	1187
GCCAGACCGG CTGGTGGAGA AACAGGAGAC AATCTGGGGA CTGAACCTTA CCCAGGGCTG	1247
TAGGAGTGAG ACTCTGAATA AAGGGTTGGG CCGGCAAAAA AAAAAAAAAA AAAAAAAAAA	1305

-24-

TABLE 5

**COMPARISON OF AMINO ACID SEQUENCES OF MONO-ADP-
RIBOSYLTRANSFERASES FROM RABBIT AND HUMAN**

A: mono-ADP-ribosyltransferase from rabbit

B: residues 51-274 of mono-ADP-ribosyltransferase from human

The character to show that two aligned residues are identical is '|'

A	- MWVPAVANLLLLSLGLLEAIQAQSHLVTRRDLFSQETPLDMPASFDDQY	-50
A	- VGCAAAMTAALPHLNLTEFQVNKVYADGWALASSQWRERSAWGPEWGLST	-100
B	- VGCAAAMTAALPDLNHTFQANQVYADSWTLASSQWQERQARWPEWSLSP	-50
A	- TRLPPPPAGFRDEHGVALLAYTANSPLHKEFNAAVRQAGRSRAHYLQHFS	-150
B	- TRPSPPLGFRDEHGVALLAYTANSPLHKEFNAAVREAGRSRAHYLHHFS	-100
A	- FKTLHFLLTEALQLLGRDQRMPCRQVFRGVHGLRFRPAGPGTTVRLGGF	-200
B	- FKTLHFLLTEALQLLGSGQRP RCHQVFRGVHGLRFRPAGPRATVRLGGF	-150
A	- ASASLKNVAAQQFGEDTFFGIWTC LGVP IQGYSFFPGEEEEVLIPPFETFQ	-250
B	- ASASLKHVAAQQFGEDTFFGIWTC LGAPIKGYSFFPGEEEEVLIPPFETFQ	-200
A	- VINASRPAQGPARIY LKALGKRSSYNCEYIKEMQCKSRPCHLDNSASAQE	-300
B	- VINASRPAQGPARIYLRALGKHST	-224
A	- RLSTAWSLLLLLAFLAVGPFPGSPGLF	-327

Identity : 195 (87.1%)

Number of gaps inserted in A: 0

Number of gaps inserted in B: 0

-25-

Diagnostic Tests to Assess the Presence or Absence of ADP-ribosyltransferase Transcripts in Cell Preparations.

Northern Blots are used to detect the presence of ADP-ribosyltransferase specific transcripts in cell samples from a patient. Tissue biopsies are obtained from a patient, washed briefly in sterile saline and lysed in guanidine isothiocyanate. RNA is isolated from the lysate using commercially available kits such as the RNA isolation kits available from Invitrogen. Purified total RNA or oligo (dT) column purified mRNA is blotted onto nylon membranes in a range of from 0.05 μ g to 5 μ g per blot. Probes complementary to the human ADP-ribosyltransferase gene sequence such as primers 5-1 and 5-2 (see Example 11) are end labelled with ^{32} P using polynucleotide kinase (Pharmacia) or commercially available kits. The probes are hybridized to the blotted RNA using conditions provided in Example 6 and developed at -80°C using Kodak X-Omat film. Developed spots indicate the presence of human ADP-ribosyltransferase transcripts.

Gene Therapy using the mono-ADP-ribosyltransferase

It is contemplated that the human sequence encoding mono-ADP-ribosyltransferase can be used in a number of gene therapeutic strategies recognized in the art. For example, the full length sequence or a portion of the sequence encoding an enzymatically active fragment is incorporated into a suitable gene delivery vehicle. There are a number of gene delivery vehicles recognized in the art that are useful for delivering a gene sequence to a cell. RNA and DNA gene sequences can be incorporated into viral vectors such as retroviral vectors, influenza vectors and adenovirus vectors. Similarly, RNA and DNA gene sequences can be introduced to cells in vivo as naked gene sequences or associated with membrane fusion promoting agents such as Lipofectin[®], or the like.

Introduction of the gene into patients in need of increased levels of mono-ADP-ribosyltransferase can be

-26-

accommodated by in vitro gene therapy. Samples of patient cells are removed and digested into single cell suspensions. The single cell suspension is then transfected with the mono-ADP-ribosyltransferase gene that is incorporated into a suitable mammalian expression vector such as those available from Stratagene, La Jolla, California; New England Biolabs, Beverly Mass; or Promega, Madison, Wisconsin. The expression vectors preferably contain suitable promoters such as an SV40 promoter, the Cytomegalovirus immediate early promoter, or the like, as well as a selection mechanism such as thymidine kinase or neomycin. Selection of transformants in vitro is followed by the re-introduction of the cells into, preferably, the same patient in need of increased levels of mono-ADP-ribosyltransferase.

It is additionally contemplated that antisense molecules may be prepared from the gene sequence and introduced into cells in need of ADP-ribosyltransferase down-regulation. Antisense technology is known in the art, for detailed applications of antisense technologies see U.S. Patent No. 4,948,882 to Ruth and European Patent Publication no. EP-387775 to Beug, et al.

Mutagenesis of NAD: Arginine ADP-ribosyl transferase

The identification of the gene sequence in mammals and humans facilitates further structure/function studies to assess the interaction of the enzyme with proteins within the cell. Since the ADP-ribosyltransferases are localized within different cellular compartments, it will be possible to modify the cellular targeting of the transferase gene, through in vitro mutagenesis, and thereby alter the localization of the expressed protein and its contact with cell substrates. Transfection of mammalian cells is currently being performed in the laboratory both with intact sequence and sequence subjected to mutagenesis.

There are a variety of commercial kits available for generating site-directed mutants or random mutants of the ADP-

-27-

ribosyltransferase (Bio-Rad, Richmond, California, Stratagene and Invitrogen, San Diego, California). Once the nucleic acid sequence is incorporated into a suitable vector, the sequence is modified by oligonucleotides containing the random or site-directed mutation. Incorporation of the oligonucleotide into the unmodified sequence may occur by PCR, ligase chain reaction, single-strand mutagenesis or the like. Mutagenesis techniques are well known in the art and commercially available as kits from Bio Rad, Invitrogen, and Stratagene. These kits include extensive directions and protocols therefore no further detail is necessary to enable one with skill in the art of molecular biology to use the sequences provided herein to generate mutation in the ADP-ribosyltransferase gene.

Generation of Antibodies

ADP-ribosyltransferase gene sequence incorporated into a eukaryotic or prokaryotic expression vector is useful for generating large quantities of the enzyme that cannot otherwise be harvested easily from vertebrate tissue. Large quantities of the enzyme are useful for crystallography, for in vitro enzyme studies and for antibody preparation.

Example 11 provides methods for generating microgram/ml quantities of the enzyme that are suitable for immunization. Mice, rats or rabbits are immunized and boosted with the enzyme preparation in the presence of a suitable adjuvant such as complete or incomplete Freund's adjuvant. Polyclonal antibodies prepared by the method of Example 12 and monoclonal antibodies prepared from the methods of Example 13 are used for diagnostic assays to assess the presence of the enzyme within a cell sample. Antibodies reactive with the enzyme permit the generation of enzyme linked immunosorbent assays (ELISA), western blots, and radioimmunoassays or the like. Example 13 details the production of an ELISA assay to detect the presence of ADP-ribosyltransferase in a cell sample.

-28-

Identification of other NAD: Arginine ADP-ribosyltransferase from other vertebrates:

It is contemplated that the methods disclosed herein are suitable for the isolation and sequence identification of mono-ADP-ribosyltransferase from any vertebrate. Tissue homogenates can be used to isolate intact enzyme that is purified and subjected to tryptic digestion to identify the amino acid sequence. Alternatively, RNA isolated from tissue homogenates is useful for direct identification of the ADP-ribosyltransferase sequence using degenerate primers in PCR reactions as disclosed for the human ADP-ribosyltransferase sequence.

Particular embodiments of the invention will be discussed in detail and reference will be made to possible variations within the scope of the invention. There are a variety of alternative techniques and procedures available to those of skill in the art which would similarly permit one to successfully perform the intended invention.

Example 1
Purification of ADP-ribosyltransferase

Frozen rabbit skeletal muscle (1 kg, Pel-Freeze, Rodgers, Arkansas) was thawed, ground and homogenized in a Waring blender for 1 minute at 4°C in 3 liters of buffer A (10% sucrose/ 10 mM histidine, pH 7.0/ 1 mM EDTA/ 1mM benzamidine/ 1mM iodoacetamide/ 0.25 mM PMSF/leupeptin, pepstatin and aprotinin, each 0.5 µg/ml, Sigma, St. Louis, Missouri). The homogenate was centrifuged at 15,000g for 30 minutes and the resulting supernatant was centrifuged at 100,000g for 2 hours. The pellet containing 1.5 g of protein as determined by BCA protein quantitation assay (Pierce Biochemicals, Rockford, Illinois) was washed once with 400 ml of buffer B (0.6 M KCl/10mM histidine, pH 7.0/1mM EDTA/ 1 mM benzamidine/ 1mM iodoacetamide/ 0.25 mM PMSF/ leupeptin, pepstatin and aprotinin, each 0.5 µg/ml) and centrifuged at 100,000g for 1 hour. The pellet (1.4 g of protein) was suspended in 200 ml

-29-

of buffer A supplemented with 0.3% sodium deoxycholate (Sigma), stirred for 30 minutes at 4°C and centrifuged at 100,000g for 2 hours. The supernatant, containing 0.6 g of protein was applied to a column (5 x 55 cm) of DE52, equilibrated with buffer C (10 mM potassium phosphate, pH 7.5/10% glycerol/0.05% sodium deoxycholate/ 1mM EDTA/ 1mM benzamidine).

The column was washed and eluted with a linear gradient of 0-1 M NaCl in buffer C (total volume 4 liters; flow rate 6 ml/min; 20-ml fractions). Transferase activity was eluted as a single peak with maximal activity at 0.4 M NaCl. Active fractions were pooled and applied to a column (1.4 x 4 cm) of concanavalin A agarose (Sigma) equilibrated with buffer D (50 mM Tris-Cl, pH 7.5/0.2 M NaCl/ 1% CHAPS/ 0.01% NaN₃), followed by washing with buffer D and eluted with 25 ml of buffer D plus 0.3 M methylmannopyranoside. The eluate was dialyzed at 4°C against buffer E (10mM Tris-Cl, pH 7.5, 1%CHAPS/0.01% NaN₃) and applied (4 ml/min) to a high resolution DEAE column (MemSep® cartridge, 1.4 ml bed volume, Millipore, Medford, Massachusetts), previously equilibrated with buffer E. After washing with buffer E, the column was eluted with a linear gradient of 0-0.3 M NaCl in 60 ml of buffer E (flow rate 2 ml/min). Four 20ml fractions that eluted at 0.025 to 0.075 M NaCl and contained transferase activity were pooled and concentrated to 0.8 ml (Centricon 30 microconcentrators, Amicon, Beverly, Massachusetts). The resulting solution was loaded successively in 200-μl samples onto a TSK 3000 HPLC gel filtration column (TosoHaas, Philadelphia, Pennsylvania). The column was eluted with buffer F (50 mM Tris-Cl, pH 7.0/ 0.2 M NaCl/ 1% CHAPS/ 0.01% NaN₃) at a flow rate of 0.9 ml/min and 0.45-ml fractions were collected. 15-μl samples of fractions 31-41 were analyzed by SDS-PAGE in 10% acrylamide gel. Samples obtained from the HPLC gel filtration column were passed through a gel filtration column a second time in the presence of 1% SDS. 20-μl aliquots of fractions 27-35 that had passed through two gel filtration columns were analyzed by

-30-

SDS-PAGE in a 12% acrylamide gel. The electrophoretic profile of the gel filtration HPLC-purified ADP-ribosyltransferase samples indicated that the second HPLC purification was useful in obtaining essentially pure ADP-ribosyltransferase. Fractions 37 and 38, containing the peak of transferase activity, were subjected to SDS-PAGE (without reducing agent). The lane corresponding to fraction 32 from the second polyacrylamide gel contained 0.1 μ g of protein. Both gels were silver stained according to the methods of Rabilloud, et al. *Electrophoresis* 9: 288-291, 1988. The gel was sliced into 2-mm fragments and proteins were eluted by shaking the slices overnight at room temperature in 50 mM Tris-Cl, pH 7.5 with 1% CHAPS. Transferase activity was found in slices corresponding to the 38-kDa protein band as identified by the kDa markers used in the polyacrylamide gels. Most of the high molecular weight contaminating protein was removed by reloading the fractions containing transferase activity on the same HPLC column and eluting with buffer F plus 1% SDS. Before assaying the fractions, SDS was removed by precipitation with 0.2 M potassium phosphate followed by repeated concentration and dilution with buffer lacking SDS using a Centricon 30 microconcentrator.

Example 2

Amino Acid Sequence Analysis of the ADP-ribosyltransferase

Proteins present in fractions 37 and 38 from the HPLC gel filtration protocol, in the absence of SDS, were separated by SDS-PAGE in a 10% gel and transferred to PVDF membrane. The band corresponding to the transferase (38 kDa, 10 μ g of protein) was excised and subjected to in situ tryptic digestion. Peptides were HPLC-purified and seven were sequenced (Harvard Microchemistry Facility, Dr. William Lane). The procedure followed the protocol of Aebersold, et al. *Proc. Natl. Acad. Sci. USA* 84: 6970-6974, 1987. The yield of the amino acids detected in each cycle ranged from 60 pmol in early cycles to 1 pmol in later cycles. The sequences of the

5 Generation of Partial ADP-ribosyltransferase Sequences by
PCR

The product from the first amplification (1 μ l) was used as a template in a second round of PCR, together with B2 primer (SEQ ID NO:14) 50 pmol, 5' to B3 and B4 (SEQ ID NOS:15 and 16, respectively) and BSC2 primer (SEQ ID NO:18, 10 pmol, pBluescript specific, 3' to BSC1, SEQ ID NO:17). Amplification conditions were the same except for the annealing temperature, which was raised to 63°C. The major product (330-bp) was subcloned into a TA cloning plasmid vector (Invitrogen, San Diego, California) and sequenced by the Sanger dideoxy chain termination method using the Sequenase sequencing kit using deoxyadenosine 5' [α -³⁵S]

Sequenase sequencing kit using deoxyadenosine 5' [α - ^{35}S]

-32-

thiotriphosphate ($^{1233}\text{Ci}/\text{mmol}$, NEN-DuPont).

Example 4
Screening of the cDNA Library

5 A Lambda ZAPII rabbit skeletal muscle cDNA library (Stratagene, 1.7×10^{10} pfu/ml) was screened in *E. coli* XL-1 Blue host cells (Stratagene) by plaque hybridization (about 5×10^5 plaques) with the 48SP oligonucleotide probe, SEQ ID NO:27, labeled with [α - ^{32}P] dATP (New England Nuclear, Beverly, Massachusetts) and terminal deoxynucleotidyl transferase (GIBCO-BRL, Gaithersburg, Maryland) to a specific activity of 5×10^7 cpm/pmol. Duplicate lifts of 2 minutes and 4 minutes were performed using nylon colony/plaque hybridization filters. Filters were prehybridized for 4 hours at 42°C in 5xSSC (1xSSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 5x Denhardt's solution (1x = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02 % bovine serum albumin), 10 mM Tris-Cl (pH 7.4), 10% dextran sulfate, 0.5% SDS and salmon sperm DNA (100 $\mu\text{g}/\text{ml}$, Lofstrand Laboratories, Gaithersburg, Maryland). Hybridization was performed in the same solution, supplemented with radiolabeled probe (2×10^6 cpm/filter). Filters were washed twice in 2xSSC/0.5% SDS at room temperature and twice in 0.5xSSC/0.5% SDS at 42°C and exposed to Kodak X-OMAT film for 24 hours at -80°C with intensifying screens. After three rounds of screening, several positive clones were identified. pBluescript plasmids carrying the cloned cDNA insert were excised in vivo, purified, and sequenced.

30

Example 5
Rapid Amplification of 5'-end of cDNA (5'-RACE)

Amplification was performed as described (Frohman, et al. *Proc. Natl. Acad. Sci. USA* 85: 8998-9002 1988 with some modifications. Poly (A)⁺ RNA from rabbit skeletal muscle was denatured with methyl mercury hydroxide and the first cDNA

35

-33-

strand was synthesized by extension of primer TG, SEQ ID NO:19 (Table 2) with AMV reverse transcriptase (Invitrogen). After 3'-end tailing of the product with dATP and terminal deoxynucleotidyl transferase, the second cDNA strand was synthesized by annealing and extending primer R₀R₁T, SEQ ID NO:21 with Taq DNA polymerase. Two rounds of PCR amplifications were then performed using primers TG, SEQ ID NO:19 and CAU-AC, SEQ ID NO:20, on one side of the cDNA fragment and R₀, SEQ ID NO:22 and CUA-R₁, SEQ ID NO:23 on the other side. The final product was subcloned into the pAMPI vector using the CloneAmp system (GIBCO-BRL) and sequenced.

Example 6 Northern Blot Analysis

Total RNA was isolated from rabbit tissues as described by Chomczynski and Sacchi (*Anal. Biochem.* 162: 156-159, 1987). Poly (A)⁺ RNA was purified from total RNA using oligo(dT) columns (Clontech, Palo Alto, California). For Northern blot analysis, 20-30 µg of total RNA or 5 µg of poly(A)⁺ RNA was subjected to electrophoresis in a denaturing 1.2% agarose gel containing formaldehyde and ethidium bromide and then transferred to Nytran membrane. After prehybridization for 12 hours at 42°C in 5xSSC/10xDenhardt's reagent/ 40% formamide/ 0.1% SDS/ 10% dextran sulfate/ and 100 µg/ml of salmon sperm DNA, hybridization was performed for 16 hours at 42°C in 5xSSC/ 2xDenhardt's/ 40% formamide/ 3% SDS/ 10% dextran sulfate/ 100 µg/ml of salmon sperm DNA and an oligonucleotide probe (2x10⁶ cpm/ml), radiolabeled as described in Example 4. Blots were washed twice in 2xSSC/ 0.1% SDS and once in 0.5xSSC/ 0.1% SDS at room temperature and once in 0.1xSSC/ 0.1% SDS at 60°C and exposed to Kodak X-OMAT film at -80°C for 24 hours with intensifying screens. Transferase specific probe 5PRM, SEQ ID NO:26 (see Table 2 for probe sequence) was specific to the 5'-untranslated region of the cDNA; Probe 48SP, SEQ ID NO:27, was specific to the coding region; and probe 3PRM, SEQ ID NO:28, was specific to the 3'-end of the

-34-

coding region.

This procedure was also used to assess the distribution of ADP-ribosyltransferase specific RNA in different tissues. These tissues included skeletal muscle, smooth muscle, heart, brain, lung, kidney, spleen and liver. 20-30 μ g of total RNA from the indicated tissues were hybridized with the transferase-specific probe, 48SP, SEQ ID NO:27. Total RNA was visualized on the gel following ethidium bromide staining using UV transillumination.

10

Example 7

Expression of ADP-ribosyltransferase in *E. coli*

ADP-ribosyltransferase cDNA was amplified by PCR using the primers 5NdeI, SEQ ID NO:24 and 3BamHI, SEQ ID NO:25. The PCR product was gel-purified, digested with *NdeI* and *BamHI* restriction enzymes (Promega) and the resulting 875-bp fragment was ligated to *NdeI*- and *BamHI*-digested pET3a (Novagen) with T4 DNA ligase (Promega) at 16°C for 16 hours. BL21(DE3) cells (Novagen) were transformed with the ligation product and applied to LB/ampicillin plates. After incubation overnight at 37°C, colonies were screened by hybridization with the 48SP oligonucleotide probe, SEQ ID NO:27. One positive colony was grown at 37°C for 4 hours in LB/ampicillin medium. The culture was then diluted 1:10 in 5 ml of the same medium, grown for 1 hour and induced with 0.4 mM IPTG (isopropyl- β -D-thiogalactopyranoside) for 1.5 hours. After centrifugation at 10,000g for 2 minutes the pellet was dispersed in 10 mM Tris-Cl, pH 8.0/ 1 mM EDTA/ 0.5 mM PMSF/ leupeptin, aprotinin and pepstatin, each 0.5 μ g/ml. Following a 30s sonication on ice, samples were used for SDS-PAGE or transferase assay.

Protein concentration was determined either by BCA assay or ISS protein gold (Integrated Protein Systems, Natick, Massachusetts) with bovine serum albumin as the standard. SDS-polyacrylamide gels were stained with Coomassie Blue or with silver stain (Rabilloud, et al., supra).

35

-35-

Example 8

Expression of ADP-ribosyltransferase in mammalian cells

5 Rat mammary adenocarcinoma (NMU) cells were grown in Eagle's Modified Essential Medium (EMEM) containing 10 % fetal calf serum. Subconfluent NMU cells on 100 x 20 mm dishes were transformed with 15 μ g of purified pMAMneo (Higuchi, (1989) in PCR Technology: Principles and Applications for DNA Amplification, Ehrlich, H. A., ed., pp. 61-70, Stockton press, New York), pM-T, pM-AT or pM-3'T constructs by the calcium phosphate precipitation method (Ausubel et al., (1990) Current Protocols in Molecular Biology, Vol. I, p. 9.1.1., John Wiley & Sons, New York).

15 To generate the pM-T construct, *NheI* and *XhoI* restriction sites were added to the 5' and 3' ends, respectively, of the rabbit skeletal muscle ADP-ribosyltransferase cDNA during PCR amplification for ligation into pMAMneo. The PCR product and pMAMneo vector were digested with *NheI* and *XhoI* and ligated using T4 DNA ligase. In the pM-AT construct, the ADP-ribosyltransferase cDNA was ligated into the pMAMneo vector in the reverse orientation. To generate the pM-3'T construct, the truncated form of the ADP-ribosyltransferase, from which 75 bases were removed at the 3'-end of the cDNA coding region, was cloned into pMAMneo. All cloning steps were methods well known in the art.

25 Cells were allowed to double before plating in selective medium (EMEM containing 10% FCS and 500 μ g/ml G418). Expression of stably incorporated ADP-ribosyltransferase was induced by incubation of cells with 1 μ M dexamethasone sodium phosphate for 48 hours (Sardet et al., (1989) Cell, 56: 271-280).

Example 9

Assay to Detect ADP-ribosyltransferase Activity

35

ADP-ribosyltransferase activity was assayed in 300 μ l of 50 mM potassium phosphate, pH 7.5, with 20 mM agmatine (Sigma,

-36-

St. Louis, Missouri) and 0.1 mM [adenine-U-¹⁴C]NAD (1.7 mCi/mmol) (Amersham, Arlington Heights, Illinois) and cold NAD (Sigma). After incubation at 30°C, a 100-μl sample was applied to a 1-ml column of Dowex AG 1-X2 (Bio-Rad, Richmond, California). [14C]ADP-ribosylagmatine was eluted with 5 ml of H₂O for radioassay. The elution profiles of [14C]ADP-ribosylagmatine with 0.1 M sodium phosphate, pH 4.5 (flow rate 1 ml/minute) after incubation with native transferase, recombinant enzyme or control *E. coli* cells, transformed with expression vector lacking insert, with 0.1 mM [adenine-U-¹⁴C]NAD or without or with 20 mM agmatine. The elution times for adenosine, nicotinamide (Nic) and NAD did not vary between the native and recombinant enzyme.

Example 10
Identification of the Human
mono-ADP-ribosyltransferase sequence

Human skeletal muscle mRNA (0.5μg, Clontech) was reverse transcribed (Invitrogen) using a mixed oligo(dT) primer (0.2 μg) and random hexamer primers (1 μg) (total volume 20 μl) (Invitrogen). Techniques for isolating mRNA are disclosed in Example 6. The first strand of cDNA was used as a template in a PCR reaction employing mixed primers 1A, 1B and 2, SEQ ID NOS:5-7, (50 pmol of each). Amplification products (1 % of the reaction volume) were reamplified in a second PCR reaction, using primers 3 and 4, SEQ ID NOS 8 and 9, respectively (50 pmol of each). Both PCR amplifications were performed under the same conditions (35 cycles of 94°C for one minute, 72° for two minutes; followed by extension at 72° for 7 minutes).

Example 11
Completion of the Human ADP-ribosyltransferase Sequence

The fragment of human ADP-ribosyltransferase as provided in Table 5 and SEQ ID NO:3 consisted of 224 amino acids. The full length nascent protein is likely to be about 330 amino acids long. It was estimated that about 70% of the human

-37-

sequence was known and about 30% of the sequence still remained to be identified. The 5' and 3' remaining portions of the sequence were identified using 5'-RACE and 3'-RACE methods (rapid amplifications of 5'-end and 3'-end of cDNA, respectively) see Frohman, et al. *Technique- A Journal of Methods in Cell and Molecular Biology* 1: 165-170 (1989). These procedures are easily performed by those skilled in the art and are used routinely in our laboratory. One example of the 5'-RACE methodology is provided in Example 5.

Human skeletal muscle poly(A)+ RNA (1 μ g) was denatured with methylmercury hydroxide and reverse transcribed with MoMLV reverse transcriptase and 100 ng of transferase-specific primers; HSM-5 for 5'-RACE (SEQ ID NO: 29), and R₀ primer (SEQ ID NO: 22) for 3'-RACE.

The first cDNA strand product from the 5' end was incubated with dATP and terminal deoxynucleotidyl transferase to add a 3' deoxyadenosine tail as described (Frohman and Martin, 1989). The second DNA strand was synthesized using 100 ng of primers R₀ (SEQ ID NO: 22) and R₀R₁T (SEQ ID NO: 21) with Taq DNA polymerase according to the GeneAmp PCR Kit protocol (Perkin-Elmer, Norwalk, CT). Amplification was performed for 30 cycles at 94°C for 1 minute; 72°C for 2 minutes followed by a 7 minute extension at 72°C. The 50 μ l reaction mix was diluted to 1 ml with TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) and the PCR product was separated from the primers using a Centricon 100 microconcentrator (Amicon, Beverly, MA). A second amplification was performed with 1 μ l of the first amplification product as a template, and 100 ng of nested primers HSM-CAUN (SEQ ID NO: 30) and HSM-30 (SEQ ID NO: 31). Reaction conditions were the same as above. The PCR product was ethanol precipitated and 5'-phosphorylated using T4 polynucleotide kinase (1 μ l; Promega, Madison, WI) according to the manufacturer's protocol. The phosphorylated product was analyzed by electrophoresis on a low melting point 1% agarose gel, excised from the gel and subcloned into the pGEM-72(+) cloning vector (Promega, Madison, WI). Plasmid DNA

-38-

was purified and sequenced as described previously.

The first cDNA strand from the 3' cDNA end was amplified by PCR using primers R₀ (SEQ ID NO: 22) and HSM-1F (SEQ ID NO: 32). After separating the PCR product from the primers, a
5 second round of amplification was performed using primers CAUHSM-2F (SEQ ID NO: 33) and CUA-RI (SEQ ID NO: 23). Amplification conditions were identical to those for the 5'-RACE procedure, except that the reaction was continued for 35 cycles instead of 30. The amplified product was cloned into
10 the pAMP1 vector using the CloneAmp system and sequenced.

To confirm the sequence of the entire human skeletal muscle transferase cDNA, poly(A)+ RNA (1 µg) was reverse transcribed as described above using primer P-RT (SEQ ID NO: 34) followed by two rounds of PCR amplification using primers
15 HSM-1 and HSM-3 and subsequently nested primers HSM-1N (SEQ ID NO: 35) and HSM-RN (SEQ ID NO: 36). The final PCR product was subcloned and sequenced.

Example 12

Preparation of anti-ADP-ribosyltransferase antibodies

20 A truncated form of the rabbit muscle transferase lacking the hydrophobic amino- and carboxy-termini was expressed as a non-fusion protein in *E. coli* as described (Zolkiewska et al., (1992) *Proc. Natl. Acad. Sci. U.S.A.*, 89: 11352-11356). Expression of the transferase was induced with
25 isopropylthiogalactoside (IPTG) in a 20 ml suspension of *E. coli*. The suspension was sonified followed by the addition of 1% CHAPS, then centrifuged at 14,000 x g for 5 minutes. The pellet, containing 500 µg protein, was emulsified in 1 ml of PBS and 1 ml of Freund's complete adjuvant and injected
30 subcutaneously into rabbits having a body weight of 1-1.5 kg. Rabbits were injected every two weeks with transferase emulsified in Freund's incomplete adjuvant. The rabbits were bled after the fourth injection and antibody titer was assessed against ADP-ribosyltransferase on Western blots.

35

Example 13

ELISA assay to Detect the Presence
of ADP-ribosyltransferase in a Cell Sample

-39-

Techniques for generating monoclonal antibodies are well known in the art. For a review of monoclonal antibody production, selection and screening see Davis, et al. Basic Methods in Molecular Biology. 1986. Elsevier Press, New York. pp. 348-354. Briefly, the purified protein preparation (50 μ g per injection) of Example 1 or Example 7 is combined with an equal volume of complete freund's adjuvant. The remaining injections use between 20-50 μ g purified protein per injection with an equal volume of incomplete freund's adjuvant. Injections are given to the mice at weekly intervals for approximately 6 weeks.

The spleens are removed, teased and the splenocytes are isolated. Erythrocytes are lysed and the mouse splenocytes are mixed at a cell ratio of 4 spleen cells to 1 myeloma cell (cell line SP2/0, or the like, American type Culture Collection, Rockville, Maryland). 50% polyethyleneglycol is added to the cell pellet containing the myeloma and splenocytes slowly over 1 minute. This is followed with 1 ml of cell culture medium. Cells are selected in hypoxanthine, aminopterin and thymidine as described by Davis, et al. (supra). Positive colonies are screened by ELISA. Antibody produced by these methods is purified using column chromatography, ammonium sulphate cuts or other methods known in the art of immunology.

ELISA strategies are well known in the art. As one preferred example of an ELISA assay, 200 μ g of purified protein at 1 μ g/ml. in phosphate buffered saline (PBS) is incubated in each well of a 96 well ELISA plate overnight at 4°C. The wells are washed with PBS containing 0.05% Tween 20. Media from the cell fusions, mouse or patient sera is serially diluted 1:5 in PBS containing 0.05% Tween 20 and Bovine Serum Albumin (0.1 mg/ml) in serial dilutions. 200 μ l of each dilution are added in duplicate to the 96 well plate. Controls are added as well. Plates are incubated for 1 hr at room temperature and the wells are washed in PBS containing Tween, as described above. Aliquots of goat anti-mouse or

-40-

human IgG conjugated to alkaline phosphatase diluted 1:400 in PBS containing Tween is added to each well. Plates are incubated for 1 hr at room temperature. Following a wash step, 200 μ l of a suitable chromogenic substrate are added
5 with hydrogen peroxide according to directs contained in the substrate. Color development indicative of the presence of antibody to the purified protein is monitored on an ELISA reader.

10 While particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art that these embodiments are exemplary rather than limiting, and the true scope of the invention is that defined in the following claims.

-41-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: National Institutes of Health
- (ii) TITLE OF INVENTION: Mammalian Muscle NAD:Arginine
ADP-ribosyltransferase
- (iii) NUMBER OF SEQUENCES: 38
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Knobbe, Martens, Olson & Bear
 - (B) STREET: 620 Newport Center Drive, 16th floor
 - (C) CITY: Newport
 - (D) STATE: California
 - (E) COUNTRY: USA
 - (F) ZIP: 92660
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: U.S. 07/985,698
 - (B) FILING DATE: November 30, 1992
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Israelsen, Ned A.
 - (B) REGISTRATION NUMBER: 29,655
 - (C) REFERENCE/DOCKET NUMBER: NIH030.001A
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 619-235-8550
 - (B) TELEFAX: 619-235-0176

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1140 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

-42-

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 106..1086

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

GACCATCACA TGAAGCCAAC ACCAGCTCCC TTGCCCCGGA CAAGGCCTAG ATGAGGAAAG	60
TAAGAGTCAA AAGGAGAGAG AAAGTGGCCT GGGGTGGCCC CAACC ATG TGG GTT	114
Met Trp Val	1
CCT GCC GTG GCG AAT CTG CTC CTT CTG TCC CTG GGC CTT CTG GAA GCA	162
Pro Ala Val Ala Asn Leu Leu Leu Leu Ser Leu Gly Leu Leu Glu Ala	5 10 15
ATT CAG GCC CAG AGC CAC CTG GTC ACA CGT CGA GAC CTC TTC TCT CAA	210
Ile Gln Ala Gln Ser His Leu Val Thr Arg Arg Asp Leu Phe Ser Gln	20 25 30 35
GAG ACA CCG CTG GAC ATG GCC CCG GCC TCC TTT GAT GAC CAG TAC GTC	258
Glu Thr Pro Leu Asp Met Ala Pro Ala Ser Phe Asp Asp Gln Tyr Val	40 45 50
GGC TGT GCA GCA GCC ATG ACA GCT GCC CTC CCG CAT CTC AAC CTC ACG	306
Gly Cys Ala Ala Ala Met Thr Ala Ala Leu Pro His Leu Asn Leu Thr	55 60 65
GAG TTC CAG GTC AAC AAA GTG TAT GCG GAC GGC TGG GCA CTG GCA AGC	354
Glu Phe Gln Val Asn Lys Val Tyr Ala Asp Gly Trp Ala Leu Ala Ser	70 75 80
AGC CAG TGG CGG GAG CGC TCG GCC TGG GGG CCC GAG TGG GGC CTC AGC	402
Ser Gln Trp Arg Glu Arg Ser Ala Trp Gly Pro Glu Trp Gly Leu Ser	85 90 95
ACA ACC CGG CTC CCC CCG CCG CCT GCG GGA TTT CGG GAT GAA CAC GGG	450
Thr Thr Arg Leu Pro Pro Pro Pro Ala Gly Phe Arg Asp Glu His Gly	100 105 110 115
GTG GCC CTG CTG GCC TAC ACG GCC AAC AGC CCC CTA CAC AAG GAG TTC	498
Val Ala Leu Leu Ala Tyr Thr Ala Asn Ser Pro Leu His Lys Glu Phe	120 125 130
AAT GCC GCG GTA CGC CAG GCG GGC CGC TCC CGA GCC CAC TAC CTC CAG	546
Asn Ala Ala Val Arg Gln Ala Gly Arg Ser Arg Ala His Tyr Leu Gln	135 140 145
CAC TTC TCC TTC AAG ACC CTG CAC TTC CTG CTG ACC GAG GCC CTG CAG	594
His Phe Ser Phe Lys Thr Leu His Phe Leu Leu Thr Glu Ala Leu Gln	150 155 160
CTG CTG GGC AGG GAT CAG CGA ATG CCC AGA TGC CGT CAG GTG TTC CGG	642
Leu Leu Gly Arg Asp Gln Arg Met Pro Arg Cys Arg Gln Val Phe Arg	

-43-

165	170	175	
GGG GTG CAT GGA CTG CGC TTC CGG CCA GCA GGG CCC GGG ACC ACT GTC			690
Gly Val His Gly Leu Arg Phe Arg Pro Ala Gly Pro Gly Thr Thr Val			
180	185	190	195
AGG CTG GGG GGC TTT GCC TCT GCG TCA CTG AAA AAT GTA GCA GCC CAG			738
Arg Leu Gly Gly Phe Ala Ser Ala Ser Leu Lys Asn Val Ala Ala Gln			
	200	205	210
CAG TTT GGC GAG GAC ACG TTC TTT GGC ATC TGG ACC TGC CTT GGG GTC			786
Gln Phe Gly Glu Asp Thr Phe Phe Gly Ile Trp Thr Cys Leu Gly Val			
	215	220	225
CCT ATC CAG GGC TAC TCC TTT TTC CCT GGG GAG GAG GAG GTT CTG ATC			834
Pro Ile Gln Gly Tyr Ser Phe Phe Pro Gly Glu Glu Glu Val Leu Ile			
	230	235	240
CCC CCC TTT GAG ACC TTC CAG GTC ATC AAC GCC AGC AGA CCT GCC CAG			882
Pro Pro Phe Glu Thr Phe Gln Val Ile Asn Ala Ser Arg Pro Ala Gln			
	245	250	255
GGC CCT GCC CGC ATC TAC CTG AAG GCG CTG GGC AAG CGC AGC TCA TAC			930
Gly Pro Ala Arg Ile Tyr Leu Lys Ala Leu Gly Lys Arg Ser Ser Tyr			
260	265	270	275
AAC TGC GAG TAC ATC AAA GAA ATG CAG TGC AAG TCT AGG CCC TGC CAC			978
Asn Cys Glu Tyr Ile Lys Glu Met Gln Cys Lys Ser Arg Pro Cys His			
	280	285	290
CTG GAC AAT TCA GCC TCG GCT CAG GAG CGC CTC TCC ACA GCC TGG TCC			1026
Leu Asp Asn Ser Ala Ser Ala Gln Glu Arg Leu Ser Thr Ala Trp Ser			
	295	300	305
CTC CTG CTG CTG CTC GCG TTC CTT GCG GTG GGG CCC TTC CCA GGA AGC			1074
Leu Leu Leu Leu Ala Phe Leu Ala Val Gly Pro Phe Pro Gly Ser			
	310	315	320
CCA GGC CTC TTC TGACCCCCCA GACTCTGGAC ATTCCTGCCT GCTGCCTCTG			1126
Pro Gly Leu Phe			
325			
CCCACTCTGT GGAT			1140

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 327 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

-44-

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

Met	Trp	Val	Pro	Ala	Val	Ala	Asn	Leu	Leu	Leu	Leu	Ser	Leu	Gly	Leu	1	5	10	15
Leu	Glu	Ala	Ile	Gln	Ala	Gln	Ser	His	Leu	Val	Thr	Arg	Arg	Asp	Leu	20	25	30	
Phe	Ser	Gln	Glu	Thr	Pro	Leu	Asp	Met	Ala	Pro	Ala	Ser	Phe	Asp	Asp	35	40	45	
Gln	Tyr	Val	Gly	Cys	Ala	Ala	Ala	Met	Thr	Ala	Ala	Leu	Pro	His	Leu	50	55	60	
Asn	Leu	Thr	Glu	Phe	Gln	Val	Asn	Lys	Val	Tyr	Ala	Asp	Gly	Trp	Ala	65	70	75	80
Leu	Ala	Ser	Ser	Gln	Trp	Arg	Glu	Arg	Ser	Ala	Trp	Gly	Pro	Glu	Trp	85	90	95	
Gly	Leu	Ser	Thr	Thr	Arg	Leu	Pro	Pro	Pro	Pro	Ala	Gly	Phe	Arg	Asp	100	105	110	
Glu	His	Gly	Val	Ala	Leu	Leu	Ala	Tyr	Thr	Ala	Asn	Ser	Pro	Leu	His	115	120	125	
Lys	Glu	Phe	Asn	Ala	Ala	Val	Arg	Gln	Ala	Gly	Arg	Ser	Arg	Ala	His	130	135	140	
Tyr	Leu	Gln	His	Phe	Ser	Phe	Lys	Thr	Leu	His	Phe	Leu	Leu	Thr	Glu	145	150	155	160
Ala	Leu	Gln	Leu	Leu	Gly	Arg	Asp	Gln	Arg	Met	Pro	Arg	Cys	Arg	Gln	165	170	175	
Val	Phe	Arg	Gly	Val	His	Gly	Leu	Arg	Phe	Arg	Pro	Ala	Gly	Pro	Gly	180	185	190	
Thr	Thr	Val	Arg	Leu	Gly	Gly	Phe	Ala	Ser	Ala	Ser	Leu	Lys	Asn	Val	195	200	205	
Ala	Ala	Gln	Gln	Phe	Gly	Glu	Asp	Thr	Phe	Phe	Gly	Ile	Trp	Thr	Cys	210	215	220	
Leu	Gly	Val	Pro	Ile	Gln	Gly	Tyr	Ser	Phe	Phe	Pro	Gly	Glu	Glu	Glu	225	230	235	240
Val	Leu	Ile	Pro	Pro	Phe	Glu	Thr	Phe	Gln	Val	Ile	Asn	Ala	Ser	Arg	245	250	255	
Pro	Ala	Gln	Gly	Pro	Ala	Arg	Ile	Tyr	Leu	Lys	Ala	Leu	Gly	Lys	Arg	260	265	270	
Ser	Ser	Tyr	Asn	Cys	Glu	Tyr	Ile	Lys	Glu	Met	Gln	Cys	Lys	Ser	Arg				

-45-

	275		280		285										
Pro	Cys	His	Leu	Asp	Asn	Ser	Ala	Ser	Ala	Gln	Glu	Arg	Leu	Ser	Thr
	290					295					300				
Ala	Trp	Ser	Leu	Leu	Leu	Leu	Leu	Ala	Phe	Leu	Ala	Val	Gly	Pro	Phe
305					310					315					320
Pro	Gly	Ser	Pro	Gly	Leu	Phe									
				325											

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 669 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..669

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGC TGT GCT GCT GCC ATG ACA GCT GCT CTC CCG GAT CTC AAC CAC ACG	48
Gly Cys Ala Ala Ala Met Thr Ala Ala Leu Pro Asp Leu Asn His Thr	
1 5 10 15	
GAG TTC CAG GCC AAC CAG GTG TAT GCA GAC AGC TGG ACA CTG GCA AGC	96
Glu Phe Gln Ala Asn Gln Val Tyr Ala Asp Ser Trp Thr Leu Ala Ser	
20 25 30	
AGC CAA TGG CAG GAG CGT CAG GCC AGG TGG CCA GAG TGG AGT CTC AGC	144
Ser Gln Trp Gln Glu Arg Gln Ala Arg Trp Pro Glu Trp Ser Leu Ser	
35 40 45	
CCC ACC CGT CCA TCC CCG CCA CCC CTG GGC TTC CGC GAT GAG CAT GGG	192
Pro Thr Arg Pro Ser Pro Pro Pro Leu Gly Phe Arg Asp Glu His Gly	
50 55 60	
GTG GCC CTC CTG GCC TAC ACA GCC AAC AGC CCC CTG CAC AAG GAG TTC	240
Val Ala Leu Leu Ala Tyr Thr Ala Asn Ser Pro Leu His Lys Glu Phe	
65 70 75 80	

-46-

AAT GCA GCC GTG CGT GAG GCG GGC CGC TCC CGG GCC CAC TAC CTC CAC	288
Asn Ala Ala Val Arg Glu Ala Gly Arg Ser Arg Ala His Tyr Leu His	
85 90 95	
CAC TTC TCC TTC AAG ACA CTC CAT TTC CTG CTG ACT GAG GCC CTG CAG	336
His Phe Ser Phe Lys Thr Leu His Phe Leu Leu Thr Glu Ala Leu Gln	
100 105 110	
CTC CTG GGC AGC GGC CAG CGT CCA CCC CGG TGC CAC CAG GTG TTC CGA	384
Leu Leu Gly Ser Gly Gln Arg Pro Pro Arg Cys His Gln Val Phe Arg	
115 120 125	
GGT GTG CAC GGC CTG CGC TTC CGG CCA GCG GGG CCC CGG GCC ACC GTG	432
Gly Val His Gly Leu Arg Phe Arg Pro Ala Gly Pro Arg Ala Thr Val	
130 135 140	
AGG TTG GGG GGC TTT GCT TCT GCC TCC CTG AAG CAT GTT GCA GCC CAG	480
Arg Leu Gly Gly Phe Ala Ser Ala Ser Leu Lys His Val Ala Ala Gln	
145 150 155 160	
CAG TTT GGT GAG GAC ACC TTC TTC GGC ATC TGG ACC TGC CTT GGG GCC	528
Gln Phe Gly Glu Asp Thr Phe Phe Gly Ile Trp Thr Cys Leu Gly Ala	
165 170 175	
CCT ATC AAG GGC TAC TCC TTC TTC CCT GGA GAG GAA GAG GTG CTG ATC	576
Pro Ile Lys Gly Tyr Ser Phe Phe Pro Gly Glu Glu Glu Val Leu Ile	
180 185 190	
CCC CCC TTT GAG ACC TTC CAA GTG ATC AAT GCC AGC AGA CCG GCC CAG	624
Pro Pro Phe Glu Thr Phe Gln Val Ile Asn Ala Ser Arg Pro Ala Gln	
195 200 205	
GGC CCC GCC CGC ATC TAC CTC CGA GCC CTG GGC AAG CAC AGC ACC	669
Gly Pro Ala Arg Ile Tyr Leu Arg Ala Leu Gly Lys His Ser Thr	
210 215 220	

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 223 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly	Cys	Ala	Ala	Ala	Met	Thr	Ala	Ala	Leu	Pro	Asp	Leu	Asn	His	Thr
1				5					10					15	
Glu	Phe	Gln	Ala	Asn	Gln	Val	Tyr	Ala	Asp	Ser	Trp	Thr	Leu	Ala	Ser
			20					25						30	

-47-

Ser Gln Trp Gln Glu Arg Gln Ala Arg Trp Pro Glu Trp Ser Leu Ser
 35 40 45
 Pro Thr Arg Pro Ser Pro Pro Pro Leu Gly Phe Arg Asp Glu His Gly
 50 55 60
 Val Ala Leu Leu Ala Tyr Thr Ala Asn Ser Pro Leu His Lys Glu Phe
 65 70 75 80
 Asn Ala Ala Val Arg Glu Ala Gly Arg Ser Arg Ala His Tyr Leu His
 85 90 95
 His Phe Ser Phe Lys Thr Leu His Phe Leu Leu Thr Glu Ala Leu Gln
 100 105 110
 Leu Leu Gly Ser Gly Gln Arg Pro Pro Arg Cys His Gln Val Phe Arg
 115 120 125
 Gly Val His Gly Leu Arg Phe Arg Pro Ala Gly Pro Arg Ala Thr Val
 130 135 140
 Arg Leu Gly Gly Phe Ala Ser Ala Ser Leu Lys His Val Ala Ala Gln
 145 150 155 160
 Gln Phe Gly Glu Asp Thr Phe Phe Gly Ile Trp Thr Cys Leu Gly Ala
 165 170 175
 Pro Ile Lys Gly Tyr Ser Phe Phe Pro Gly Glu Glu Glu Val Leu Ile
 180 185 190
 Pro Pro Phe Glu Thr Phe Gln Val Ile Asn Ala Ser Arg Pro Ala Gln
 195 200 205
 Gly Pro Ala Arg Ile Tyr Leu Arg Ala Leu Gly Lys His Ser Thr
 210 215 220

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AATTTATATA TATTTATATA TTTATATTTT ATATATTTAT ATTTTATTTA 50

(2) INFORMATION FOR SEQ ID NO:6:

-48-

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AAGGAGAGAG AGGAGAGAGG GGAGGAGAGG G

31

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

NCTNGAYATG GCNCCNGC

18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

YTTRCAYTGC ATYTCYTT

18

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

NTTYGAYGAY CARTAYGT

18

-49-

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

DATRTAYTCR CARTTRTA

18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCTGTCTGCA TACACCTGGT TTGGC

25

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTCGTTGAGA TCCGGGAGAG C

21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

-50-

CCCGCATCTA CCTCCGAGCC

20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CAAGCACAGC ACCTATAATT

20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCCCCANCCAT CNGCATANAC

20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCTAANGCCC ANCCATCNGC

20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

-51-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCNAGNGCCC ANCCATCNGC

20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CAAAAGCTGG AGCTCCACCG CGGTG

25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCTCTAGAAC TAGTGGATCC C

21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGTCATGGCT GCTGCACAGC

20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

-52-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CAUCAUCAUC AUACGTACTG GTCATCAAAG GA

32

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AAGGATCCGT CGACATCGAT AATACGACTC ACTATAGGGA TTTTTTTTTT

50

TTTTTTTT

57

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AAGGATCCGT CGACATC

17

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CUACUACUAC UAGACATCGA TAATACGACT CACTATA

37

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39

-53-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTGGTTCCGG CGACATATGA GCCACCTGGT CACACGTCG

39

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CTCGCTCCGG CGAGGATCCT CAGGAGAGGC GCTCCTGAGC CG

42

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTACTTTCCT CATCTAGGCC TTGTCCGGGG CAGGGGAGCT GGTGTTGG

48

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AAAGGAGGCC GGGGCCATGT CCAGCGGTGT CTCTTGAGAG AAGAGGTC

48

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

-54-

- (A) LENGTH: 48
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGGAATGTCC AGAGTCTGGG GGGTCAGAAG AGGCCTGGGC TTCCTGGG 48

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGGTTGGTCCACATACGTCTGTCTG 24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CAUCAUCAUGTGGTTGAGATCCGGGAGAGC 33

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

ACTAGTTATGCAACCGACACGACGACGGTA 30

(2) INFORMATION FOR SEQ ID NO:32:

-55-

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CCCGCATCTACCTCCGAGCC 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CAUCAUCAUCAAGCACAGCACCTATAATT 32

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGACTCCTACAACGGGTACACACG 34

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CUACUACUACUAAGCAACTGGCCCAGGGTCACCAGC 36

-56-

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

ACGTACTCTGTGCCCTGTCAUCAUCAUCAU

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1305 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 73..1047

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TTCCACCAGG ACAGGCCTAG ATGAGGAAAC TGAGACCCAA AAAGAGACAG CAACTGGCCC	60
AGGGTCACCA GC ATG CAG ATG CCT GCT ATG ATG TCT CTG CTT CTT GTG	108
Met Gln Met Pro Ala Met Met Ser Leu Leu Leu Val	
1 5 10	
TCT GTG GGC CTC ATG GAA GCA CTT CAG GCC CAG AGC CAC CCC ATC ACA	156
Ser Val Gly Leu Met Glu Ala Leu Gln Ala Gln Ser His Pro Ile Thr	
15 20 25	
CGA CGA GAC CTC TTC TCT CAA GAG ATT CAG CTG GAC ATG GCC CTG GCC	204
Arg Arg Asp Leu Phe Ser Gln Glu Ile Gln Leu Asp Met Ala Leu Ala	
30 35 40	
TCC TTT GAT GAC CAG TAC GCT GGC TGT GCT GCT GCC ATG ACA GCT GCT	252
Ser Phe Asp Asp Gln Tyr Ala Gly Cys Ala Ala Ala Met Thr Ala Ala	
45 50 55 60	

-57-

CTC CCG GAT CTC AAC CAC ACG GAG TTC CAG GCC AAC CAG GTG TAT GCA Leu Pro Asp Leu Asn His Thr Glu Phe Gln Ala Asn Gln Val Tyr Ala 65 70 75	300
GAC AGC TGG ACA CTG GCA AGC AGC CAA TGG CAG GAG CGT CAG GCC AGG Asp Ser Trp Thr Leu Ala Ser Ser Gln Trp Gln Glu Arg Gln Ala Arg 80 85 90	348
TGG CCA GAG TGG AGT CTC AGC CCC ACC CGT CCA TCC CCG CCA CCC CTG Trp Pro Glu Trp Ser Leu Ser Pro Thr Arg Pro Ser Pro Pro Pro Leu 95 100 105	396
GGC TTC CGC GAT GAG CAT GGG GTG GCC CTC CTG GCC TAC ACA GCC AAC Gly Phe Arg Asp Glu His Gly Val Ala Leu Leu Ala Tyr Thr Ala Asn 110 115 120	444
AGC CCC CTG CAC AAG GAG TTC AAT GCA GCC GTG CGT GAG GCG GGC CGC Ser Pro Leu His Lys Glu Phe Asn Ala Ala Val Arg Glu Ala Gly Arg 125 130 135 140	492
TCC CGG GCC CAC TAC CTC CAC CAC TTC TCC TTC AAG ACA CTC CAT TTC Ser Arg Ala His Tyr Leu His His Phe Ser Phe Lys Thr Leu His Phe 145 150 155	540
CTG CTG ACT GAG GCC CTG CAG CTC CTG GGC AGC GGC CAG CGT CCA CCC Leu Leu Thr Glu Ala Leu Gln Leu Leu Gly Ser Gly Gln Arg Pro Pro 160 165 170	588
CGG TGC CAC CAG GTG TTC CGA GGT GTG CAC GGC CTG CGC TTC CGG CCA Arg Cys His Gln Val Phe Arg Gly Val His Gly Leu Arg Phe Arg Pro 175 180 185	636
GCA GGG CCC CGG GCC ACC GTG AGG CTG GGG GGC TTT GCT TCT GCC TCC Ala Gly Pro Arg Ala Thr Val Arg Leu Gly Gly Phe Ala Ser Ala Ser 190 195 200	684
CTG AAG CAT GTT GCA GCC CAG CAG TTT GGT GAG GAC ACC TTC TTC GGC Leu Lys His Val Ala Ala Gln Gln Phe Gly Glu Asp Thr Phe Phe Gly 205 210 215 220	732
ATC TGG ACC TGC CTT GGG GCC CCT ATC AAG GGC TAC TCC TTC TTC CCT Ile Trp Thr Cys Leu Gly Ala Pro Ile Lys Gly Tyr Ser Phe Phe Pro 225 230 235	780
GGA GAG GAA GAG GTG CTG ATC CCC CCC TTT GAG ACC TTC CAA GTG ATC Gly Glu Glu Glu Val Leu Ile Pro Pro Phe Glu Thr Phe Gln Val Ile 240 245 250	828
AAT GCC AGC AGA CCG GCC CAG GGC CCC GCC CGC ATC TAC CTC CGA GCC Asn Ala Ser Arg Pro Ala Gln Gly Pro Ala Arg Ile Tyr Leu Arg Ala 255 260 265	876
CTG GGC AAG CAC AGC ACC TAC AAC TGC GAG TAC ATC AAA GAC AAG AAG Leu Gly Lys His Ser Thr Tyr Asn Cys Glu Tyr Ile Lys Asp Lys Lys	924

-58-

270	275	280	
TGC AAG TCT GGG CCT TGC CAT CTG GAT AAT TCA GCC ATG GGT CAG AGC			972
Cys Lys Ser Gly Pro Cys His Leu Asp Asn Ser Ala Met Gly Gln Ser			
285	290	295	300
CCC CTC TCT GCA GTC TGG TCT TTG CTG CTG CTG CTC TGG TTC CTC GTG			1020
Pro Leu Ser Ala Val Trp Ser Leu Leu Leu Leu Leu Trp Phe Leu Val			
	305	310	315
GTG AGG GCC TTT CCA GAT GGT CCA GGC CTC CTT TGATGCATGA GACA			1067
Val Arg Ala Phe Pro Asp Gly Pro Gly Leu Leu			
	320	325	
CGGGAC AGCCTCGCCTGCTG CCTCTGCCCA TCCTGAGGAT GTTGGCCATG TGTGCTTCAG			1127
TGTAACCAAG ATTCCTGTCA ATCCCATCTG CAGGGAAGCTC TGGGACCTTC TCTGGTAGCT			1187
GCCAGACCGG CTGGTGGAGA AACAGGAGAC AATCTGGGGA CTGAACCTTA CCCAGGGCTG			1247
TAGGAGTGAG ACTCTGAATA AAGGGTTGGG CCGGCAAAAA AAAAAAAAAA AAAAAAAA			1305

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 325 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

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

-59-

Glu His Gly Val Ala Leu Leu Ala Tyr Thr Ala Asn Ser Pro Leu His
 115 120 125
 Lys Glu Phe Asn Ala Ala Val Arg Glu Ala Gly Arg Ser Arg Ala His
 130 135 140
 Tyr Leu His His Phe Ser Phe Lys Thr Leu His Phe Leu Leu Thr Glu
 145 150 155 160
 Ala Leu Gln Leu Leu Gly Ser Gly Gln Arg Pro Pro Arg Cys His Gln
 165 170 175
 Val Phe Arg Gly Val His Gly Leu Arg Phe Arg Pro Ala Gly Pro Arg
 180 185 190
 Ala Thr Val Arg Leu Gly Gly Phe Ala Ser Ala Ser Leu Lys His Val
 195 200 205
 Ala Ala Gln Gln Phe Gly Glu Asp Thr Phe Phe Gly Ile Trp Thr Cys
 210 215 220
 Leu Gly Ala Pro Ile Lys Gly Tyr Ser Phe Phe Pro Gly Glu Glu Glu
 225 230 235 240
 Val Leu Ile Pro Pro Phe Glu Thr Phe Gln Val Ile Asn Ala Ser Arg
 245 250 255
 Pro Ala Gln Gly Pro Ala Arg Ile Tyr Leu Arg Ala Leu Gly Lys His
 260 265 270
 Ser Thr Tyr Asn Cys Glu Tyr Ile Lys Asp Lys Lys Cys Lys Ser Gly
 275 280 285
 Pro Cys His Leu Asp Asn Ser Ala Met Gly Gln Ser Pro Leu Ser Ala
 290 295 300
 Val Trp Ser Leu Leu Leu Leu Leu Trp Phe Leu Val Val Arg Ala Phe
 305 310 315 320
 Pro Asp Gly Pro Gly Leu Leu
 325

-60-

What is Claimed is:

1. An assay for identifying mono-ADP-ribosyltransferase gene sequence from a vertebrate comprising:

- 5 (a) harvesting tissue containing ADP-ribosyltransferase activity from a vertebrate;
- (b) purifying said mono-ADP-ribosyltransferase from said tissue;
- (c) obtaining fragments of said mono-ADP-ribosyltransferase;
- 10 (d) sequencing peptides obtained from said fragments;
- (e) preparing degenerate oligonucleotides corresponding to the amino acid sequence of said peptides;
- 15 (f) using said oligonucleotides in at least one polymerase chain reaction to generate nucleic acid fragments from a library of nucleic acid sequences, wherein the resulting fragments correspond to at least a portion of the mono-ADP-ribosyltransferase sequence;
- 20 (g) generating the nucleic acid sequence of the polymerase chain reaction fragments;
- (h) identifying different oligonucleotides corresponding to the mono-ADP-ribosyltransferase sequence;
- 25 (i) repeating steps (f) through (h) until the complete nucleic acid sequence is identified.

2. An assay for identifying a mono-ADP-ribosyltransferase gene sequence in a vertebrate comprising:

- 30 (a) identifying tissue from said vertebrate that contains ADP-ribosyltransferase activity;
- (b) isolating mRNA from said tissue;
- (c) preparing cDNA from said mRNA;
- 35 (d) preparing an oligonucleotide pair suitable for use in a polymerase chain reaction, one oligonucleotide

-61-

of said pair having a sequence substantially the same as a first portion of SEQ ID NO: 1, and the other oligonucleotide of said pair having a sequence substantially complementary to a second portion of SEQ ID NO: 1

5

(e) performing said polymerase chain reaction on said cDNA using the oligonucleotide pair of step (d) to generate PCR-amplified fragments;

10

(f) sequencing said fragments generated from said polymerase chain reaction; and

(g) repeating steps (e) through (g) until said cDNA is completely sequenced.

15

3. An isolated or purified nucleic acid fragment encoding rabbit skeletal muscle ADP-ribosyltransferase corresponding to SEQ ID NO:1.

4. The gene sequence of Claim 3, wherein said sequence is mutated in vitro to contain at least one nucleotide change in said sequence.

20

5. Purified or isolated oligonucleotide comprising at least 15 continuous bases of SEQ ID NO:1.

6. Recombinant gene vectors containing at least 15 continuous bases of SEQ ID NO:1.

7. Recombinant protein expressed from the gene vector of Claim 6.

25

8. The recombinant protein of Claim 7, wherein said protein is essentially pure.

9. The recombinant protein of Claim 7, wherein said protein exhibits ADP-ribosyltransferase activity.

30

10. The recombinant protein of Claim 7, wherein said protein is produced in eukaryotes.

11. The recombinant protein of Claim 7, wherein said protein is produced in prokaryotes.

35

12. A gene sequence encoding vertebrate ADP-ribosyltransferase having at least an 85% homology to SEQ ID NO:1.

13. A gene sequence encoding human ADP-

-62-

ribosyltransferase consisting essentially of the sequence corresponding to SEQ ID NO:37.

14. Recombinant gene vectors containing at least 15 continuous bases of SEQ ID NO:37.

5 15. Recombinant protein expressed from the gene vector of Claim 14.

16. The recombinant protein of Claim 15, wherein said protein is essentially pure.

10 17. The recombinant protein of Claim 15, wherein said protein exhibits ADP-ribosyltransferase activity.

18. The recombinant protein of Claim 15, wherein said protein is produced in eukaryotes.

19. The recombinant protein of Claim 15, wherein said protein is produced in prokaryotes.

15 20. Purified antibody capable of specifically binding to the recombinant protein encoded by SEQ ID NO:1.

21. Purified antibody capable of specifically binding to the recombinant protein encoded by SEQ ID NO:37.

20 22. An assay for detecting an ADP-ribosyltransferase gene sequence homologous to SEQ ID NO:37 in a vertebrate comprising the steps of:

25 (a) obtaining at least one oligonucleotide pair from SEQ ID NO:37 suitable for a polymerase chain reaction and a third oligonucleotide selected from SEQ ID NO:3 positioned between said oligonucleotide pair;

(b) isolating a tissue sample from said vertebrate;

30 (c) processing said tissue to obtain nucleic acid suitable as a template for use in a polymerase chain reaction;

(d) performing a polymerase chain reaction using said oligonucleotide pair to generate at least one DNA fragment;

35 (e) hybridizing said third oligonucleotide with said DNA fragment; and

(f) detecting hybridization between said third

-63-

oligonucleotide and said DNA fragment.

23. An assay for detecting ADP-ribosyltransferase in a vertebrate, comprising the steps of:

5 (a) obtaining purified antibody capable of specifically binding to SEQ ID NO:1;

(b) exposing said antibody to a tissue or fluid sample from said vertebrate; and

10 (c) detecting binding between said antibody and said tissue or fluid sample, wherein said binding indicates the presence of ADP-ribosyltransferase in said sample.

1/1

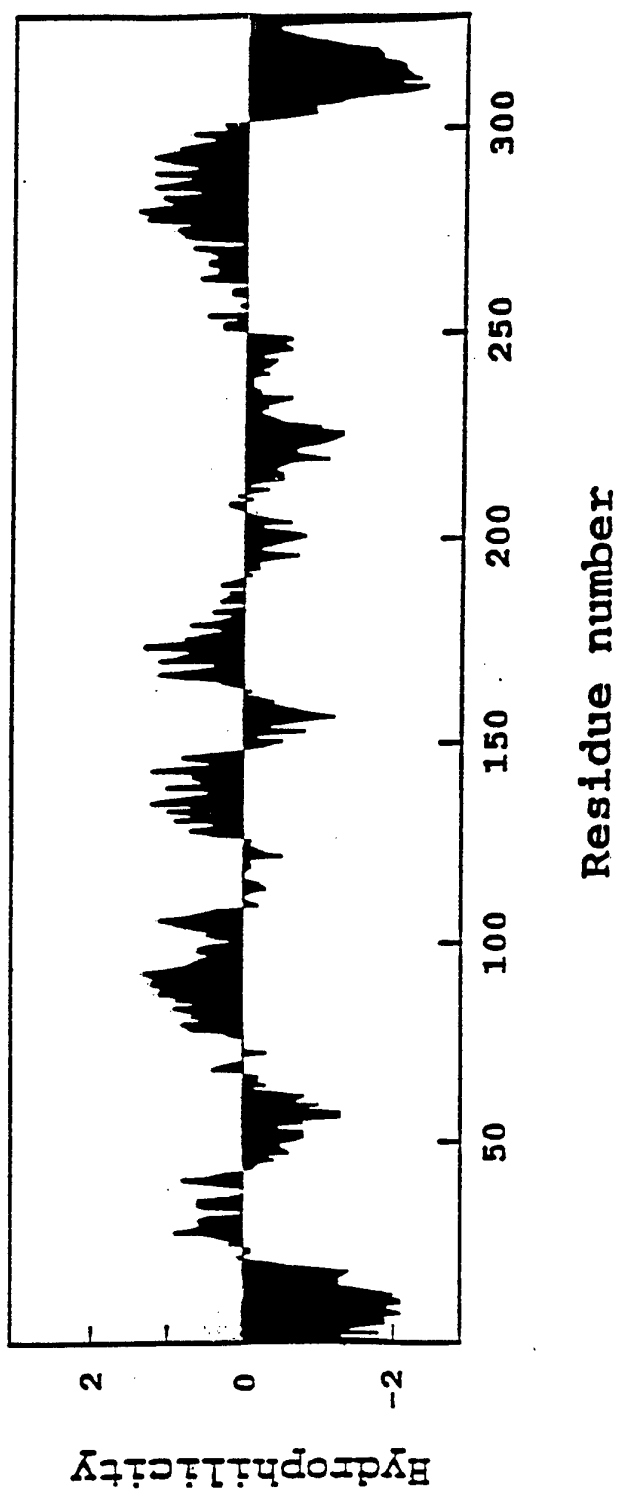


FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11569

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C12Q 1/68, 1/48; C12N 15/63, 15/54, 9/12; C07H 15/12;

US CL : 435/ 6, 7.1, 194, 320.1; 536/23.2; 530/388.26

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/ 6, 7.1, 194, 320.1; 536/23.2; 530/388.26

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

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

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Biol. Chem., Vol. 265, No. 28, issued 05 October 1990, Peterson et al., "Purification And Partial Characterization Of Arginine-Specific ADP-ribosyltransferase From Skeletal Muscle Microsomal Membranes", pages 17062-17069, see entire document.	1-12, 20, 23
Y	US, A, 4,289,690 (PESTKA ET AL.) 15 September 1981, see entire document.	7-11
Y	J. D. WATSON, "MOLECULAR BIOLOGY OF THE GENE", 3rd. edition, published 1987, by The Benjamin/Cummings Publishing Company, Inc. (CA), page 313, see page 313.	1-12, 20, 23



Further documents are listed in the continuation of Box C.



See patent family annex.

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

Date of the actual completion of the international search

25 FEBRUARY 1994

Date of mailing of the international search report

MAR 11 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

CHRISTOPHER S. F. LOW

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/US93/11569

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,675,285 (CLARK ET AL.) 23 June 1987, see entire document.	1-12, 20, 23
Y	Technique, Vol. 1, No. 3, issued December 1989, Frohman et al., "Rapid Amplification Of cDNA Ends Using Nested Primers", pages 165-170, see entire document.	1-6, 12
Y	Proc. Natl. Acad. Sci. USA, Vol. 85, issued December 1988, Frohman et al., "Rapid Production Of Full-Length cDNAs From Rare Transcripts: Amplification Using A Single Gene-Specific Oligonucleotide Primer", pages 8998-9002, see entire document.	1-6, 12
Y	US, A, 4,683,195 (MULLIS ET AL.) 28 July 1987, see entire document.	1-6, 12
Y	J. Biol. Chem., vol. 258, no. 8, issued 25 April 1983, Yost et al., "Amino Acid-specific ADP-ribosylation. Evidence For Two Distinct NAD:Arginine ADP-ribosyltransferases In Turkey Erythrocytes", pages 4926-4929, see entire document.	1-12, 20, 23
Y	Proc. Natl. Acad. Sci. USA, Vol. 86, issued May 1989, Herzog et al., "Human Nuclear NAD ⁺ ADP-ribosyltransferase: Localization Of The Gene on Chromosome 1q41-q42 And Expression Of An Active Human Enzyme in <i>Escherichia coli</i> ", pages 3514-3518, see entire document.	1-12, 20, 23
Y	Eur. J. Cell Biol., Vol. 44, issued 1987, Schneider et al., "Isolation of a cDNA clone For Human NAD ⁺ :Protein ADP-ribosyltransferase", pages 302-307, see entire document.	1-12, 20, 23
P, <u>X</u> Y	Proc. Natl. Acad. Sci. USA, Vol. 89, issued December 1992, Zolkiewska et al., "Molecular Characterization Of NAD ⁺ :arginine ADP-ribosyltransferase From Rabbit Skeletal Muscle", pages 11352-11356, see entire document.	<u>3-12</u> 1-12, 20, 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11569

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 13-19, 21 and 22
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

(Telephone Practice)

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/11569

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

Automated Patent System - USPAT and JPOABS

Dialog - files 5, 399, 144, 434, 357, 155, 156, 76, 35, 149, 340

Search terms: adenine, adenosine, adp, nad, dinucleotide, phosphate, phosphorous, ribose, ribosyl, ribosyltransferase, ribosylation

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

Claims 13-19, 21 and 22 require a search of the sequence defined by SEQ ID NO: 38. The submitted computer readable sequence as filed is defective and not available for search purposes. Claims requiring a search using SEQ ID NO: 1 were searched using the data submitted in the priority document for the claims 1-12, 20, and 23. The priority document contains no sequence defined as SEQ ID NO:38.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This international application contains the following inventions or groups of inventions which are not so linked by the identical special technical features so as to form a single inventive concept under the criteria of PCT Rule 13.2 as set forth below in Groups I through IV.

- I. Claims 1-6 and 12, drawn to the first process, an assay to identify/obtain DNA encoding mono-ADP-ribosyltransferase, and the first set of products which are the vectors and DNA encoding the enzyme are for example, classified in Class 435, subclasses 6, 320.1 and Class 536, subclass 23.2.
- II. Claims 7-11, drawn to a second set of products consisting of the enzyme mono-ADP-ribosyltransferase are for example, classified in Class 435, subclass 194.
- III. Claim 20, drawn to the third product of an antibody to the enzyme is for example, classified in Class 530, subclass 388.26.
- IV. Claim 23, drawn to a second process which is detecting the presence of the enzyme mono-ADP-ribosyltransferase is for example, classified in Class 435, subclass 7.1.