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(54) Title: DNA ENCODING THE HUMAN SYNAPSIN III GENE AND USES THEREOF		
(57) Abstract A new synapsin protein, designated synapsin III, its amino acid sequence, and its human gene have been isolated and characterized. Furthermore, isoforms of synapsin III, e.g., synapsin IIIa, IIIb and IIIc, and have been isolated and characterized, and cDNA encoding these isoforms has also been isolated and characterized. The synapsin III gene is located on human chromosome 22, in the vicinity of a region previously identified as a susceptibility locus for schizophrenia. The information and experimental tools provided by this discovery can be used to generate new therapeutic agents or diagnostic assays for this new protein, its associated mRNA or its associated genomic DNA. Due to its role in neurotransmission and synaptogenesis, isoforms of synapsin III are associated with the symptoms of psychiatric diseases, especially schizophrenia.		

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**DNA ENCODING THE HUMAN SYNAPSIN III GENE AND USES
THEREOF**

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5 certain rights therein.

CROSS REFERENCE TO RELATED APPLICATIONS

This Application is a Continuation In Part of pending Application Serial No. 08/906,865 filed on August 6, 1997, which is hereby incorporated by reference in its entirety.

10

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally isoforms of synapsin III, e.g., synapsin IIIa, IIIb, and IIIc. mRNA produced from transcription of the synapsin III gene undergoes alternative splicing and encodes the isoforms. Furthermore, the invention relates to uses of isoforms of synapsin III, and nucleic acids which encode
15 these isoforms, especially in the diagnosis and treatment of schizophrenia.

BACKGROUND OF THE INVENTION

The synapsins are a family of proteins that play a crucial role in the regulation of neurotransmission and in neurodevelopment. Single genes encode synapsins I and II. The synapsin I gene gives rise to two alternatively spliced mRNAs, which in
20 turn, give rise to the synapsin Ia and Ib protein. The synapsin II gene gives rise to two alternatively spliced mRNAs, which in turn, give rise to the synapsin IIa and IIb protein (1).

The synapsin Ia, Ib, IIa, and IIb proteins are all found in the brain, where they are specifically localized to the presynaptic region of neurons, and coat synaptic
25 vesicles. There is considerable experimental evidence that synapsins regulate the release of neurotransmission by becoming phosphorylated through the action of specific protein kinases (2).

There is also compelling evidence from several biological systems that synapsins play a critical role in axonal outgrowth, synapse formation, and synapse maintenance (3-8). Synapsins are potent stimulators of synapse formation, and may be useful as therapeutic agents for neurodegenerative diseases.

- 5 To date, no satisfactory candidate susceptibility gene for schizophrenia has been identified within the region of 22q 12-13 human chromosome 22, although several studies report that this region has a statistically significant chance of containing such a gene. Since synapsins play an integral role in neurotransmitter release and synaptogenesis, mutations in the gene therefor could explain many of the defects
10 observed in neurotransmission and in synapse formation in schizophrenia.

Schizophrenia is a psychiatric illness that affects approximately 1% of the population worldwide. The illness is characterized by positive symptoms such as hallucinations, delusions, bizarre behavior, and thought disorder, as well as negative symptoms such as lack of motivation, social withdrawal, and apathy.

- 15 Unfortunately, there is no objective laboratory test for schizophrenia, and the diagnosis is made by clinical interview. Current medication is effective for treating the positive symptoms of the disease, with little effect on the negative symptoms. Although the cause of schizophrenia is unknown, the disease has a strong genetic component. Research into the genetics of schizophrenia reveals that this disease is
20 heterogeneous and is a "complex genetic" disease - that is, several genes may be involved in the etiology of this disease. In identical twin studies, where one twin is affected by the disease, the other twin has a 50% chance of succumbing to the disease. Since identical twins possess identical genes, the penetrance of the disease in the setting of mutated genes is only 50%. Thus, an unknown environmental
25 component as well as the presence of specific mutated genes, is required for the generation of schizophrenia.

Biochemical and cytological studies suggest that in schizophrenia, defects in neurotransmission and synapses occur. Abnormal neurotransmission affecting the dopamine, serotonin, glutamine, γ -aminobutyric acid, and cholecystokinin systems have been reported in schizophrenia (18). Neurodevelopmental abnormalities are also strongly implicated in schizophrenia, with reports of defects in neuronal cytoskeleton (19), neuronal cytoarchitecture and migration (20), cellular polarity (21), and synaptic pruning (22). Thus, in schizophrenia, at least two processes appear to be aberrant: neurotransmission and neuronal development, primarily affecting the later stages of synapse formation.

10 These studies indicate that a candidate susceptibility gene for schizophrenia should be expressed in the brain, and would likely play a role in synapse formation and neurotransmission. Furthermore, a candidate susceptibility gene should be genetically linked to schizophrenia. As indicated before, several research groups have independently identified a region on chromosome 22 that appears to possess a candidate susceptibility gene for schizophrenia (9-17). To date, a susceptibility gene has not been identified in this region.

Schizophrenia is a common disease, with a world-wide prevalence of 1%, affecting families of all races and socio-economic groups, and consuming a significant portion of all medical and social expenses.

20 Since there is a continuing need for new therapies for such diseases, efforts have been devoted to the characterization and elucidation of the genes for synapsins, and their various attendant uses. The susceptibility gene for schizophrenia has great commercial value both as a diagnostic reagent and for developing new treatments for this disease.

SUMMARY OF THE INVENTION

In accordance with the present invention, novel human cDNAs which encode isoforms of synapsin III, e.g., synapsin IIIa, IIIb, and IIIc have been cloned and characterized. These cDNAs are produced from alternative splicing of RNA
5 complementary to the synapsin III gene, the newest member and third gene of the synapsin family of neuronal phosphoproteins.

This invention thus provides cDNAs of isolated clones that encode the heretofore unknown isoforms of the synapsin III protein, and which can be used to obtain genomic DNA, cDNA or RNA complementary to an isolated clone of the invention.
10 Isolated clones of the invention can be used to isolate mRNA or genomic DNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries. Furthermore, the clones themselves, or fragments derived from the clones, can be inserted in suitable vectors, such as plasmids or bacteriophages, then replicated and harvested following
15 introduction into suitable bacterial host cells. DNA or RNA fragments derived from isolated clones of the invention can also be used as probes for *in situ* hybridization in order to locate tissues which express this gene, or for other hybridization assays for the presence of the gene or an mRNA transcribed therefrom in various biological tissues. In addition, oligonucleotides complementary to the
20 sequence of an isolated clone of the invention can be synthesized and used as probes for this gene, an mRNA associated therewith, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the Polymerase Chain Reaction.

This invention may also be used to obtain isoforms of the synapsin III protein, or
25 fragments thereof, produced from expression of an isolated genomic clone. For example, an isolated clone of the invention, or fragments thereof can be subcloned into suitable expression vectors, such as the plasmid pET15b (Novagen, Madison,

Wisconsin), and transfected into suitable host cells, such as bacteria, yeast, or mammalian cells. As a result, an isoform of the synapsin III protein can be produced in the transfected host cell, and recovered from the host for direct uses or for experimental study, using methods well known in the art. Furthermore, pursuant to the invention, either an entire isoform the synapsin III protein or fragments thereof can be recovered from the transfected host for further uses.

This invention may also be used to generate antibodies directed against isoforms of human synapsin III protein. Production of an isoform, e.g., synapsin IIIa, IIIb, or IIIc, or fragments thereof, encoded by an isolated clone in transfected host cells such as those described above, would provide an isoform of the synapsin III protein, or fragments thereof, which could be used as antigen for the generation of polyclonal or monoclonal antibodies against isoforms of a synapsin III protein, using methods well known in the art. These antibodies could be used to detect the presence of an isoform of synapsin III protein in humans or animals, or in biological tissues or fluids isolated from humans or animals.

In addition, this invention may be used to isolate transcriptional or translational regulatory elements from the 5' untranslated region of the synapsin III gene, or post-transcriptional regulatory elements from the 3' untranslated regions of the isolated gene.

This invention can further be used to detect mutations in the synapsin III gene in individuals who are at risk of developing schizophrenia. Identification of these mutations will allow one to determine which individuals are at risk for developing schizophrenia.

Accordingly, it is a principal object of the present invention to provide various isoforms of the synapsin III protein and subunits or fragments thereof in purified

form that exhibit certain characteristics and activities associated with synapsin III promotion of neurotransmitter activity and synaptogenesis.

It is a further object of the invention to provide isolated nucleic acid molecules which encode various isoforms of synapsin III, e.g., synapsin IIIa, IIIb, and IIIc.

- 5 These isolated nucleic acid molecules are produced from the alternative splicing of RNA complementary to the synapsin III gene.

It is a further object of the invention to provide isolated nucleic acid molecules hybridizable under standard hybridization conditions to an isolated nucleic acid molecule that encodes an isoform of a synapsin III protein.

- 10 It is a further object of the present invention to provide antibodies to isoforms of synapsin III and their subunits, and methods for their preparation, including recombinant means.

- It is a further object of the present invention to provide a method for detecting the presence of an isoform of synapsin III and its subunits in mammals in which
15 invasive, spontaneous, or idiopathic pathological states due to neurotransmission deficiencies are suspected to be present, especially schizophrenia.

- It is a further object of the present invention to provide a method and associated assay system for screening substances such as drugs, agents and the like, potentially effective in either mimicking the activity or combating the adverse effects of an
20 isoform of synapsin III and/or its subunits in mammals.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of isoforms of the synapsin III protein, or subunits thereof, so as to alter the adverse consequences of such presence or activity, or where beneficial, to enhance such activity.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of isoforms of synapsin III or subunits or fragments thereof, so as to treat or avert the adverse consequences of invasive, spontaneous or idiopathic pathological states, especially schizophrenia and
5 other neurodegenerative diseases.

It is a still further object of the present invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon isoforms of synapsin III, or subunits thereof, their binding partner(s), or upon agents or drugs that control the production, or that mimic or antagonize the
10 activities of isoforms of synapsin III, i.e., synapsin IIIa, synapsin IIIb, or synapsin IIIc.

Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing description which proceeds with reference to the following illustrative drawings.

15 BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts schematic diagram of chromosome 22, showing the location of exons of synapsin III on cosmids localized to this gene.

FIGURE 2 is a schematic diagram of chromosome 22, showing the location of the synapsin III gene within the schizophrenia susceptibility region.

20 FIGURE 3 is a diagram illustrating the clones spanning the entire length of the Synapsin III gene.

FIGURE 4 is the cDNA sequence (upper) (SEQ ID NO: 2) and protein sequence (lower) (SEQ ID NO: 1) for synapsin IIIa.

FIGURE 5 is a comparison of the protein sequences of synapsin IIIa to synapsins
25 IIa and Ia.

FIGURE 6a is a photograph of Northern blot analysis showing the distribution of synapsin III RNA different tissues.

FIGURE 6b is a photograph of Northern blot analysis showing the distribution of synapsin III RNA in brain.

5 FIGURE 7a is a photograph of a Western blot analysis showing expression of synapsin proteins in brain.

FIGURE 7b is a photograph of a Western blot analysis showing the expression of synapsin protein in different tissues.

FIGURE 8 is the amino acid sequence of synapsin IIIb (SEQ ID NO:6).

10 FIGURE 9 is the nucleotide sequence of a nucleic acid molecule which encodes synapsin IIIb (SEQ ID NO:5).

FIGURE 10 is the amino acid sequence synapsin IIIc (SEQ ID NO:8).

FIGURE 11 is the nucleotide sequence of a nucleic acid molecule which encodes sequence of synapsin IIIc (SEQ ID NO:7).

15 DETAILED DESCRIPTION OF THE INVENTION

In its broadest aspect, the present invention extends to the isolation and characterization of an isoform of human synapsin III protein having the following characteristics:

- a) it is localized to neurons and nerve terminals; and
- 20 b) it is associated with synaptic vesicles.

In a specific example, an isoform of synapsin III of the invention comprises an amino acid sequence as shown in FIGURE 4 (SEQ ID NO:1), FIGURE 8 (SEQ ID NO:6), or FIGURE 10 (SEQ ID NO:8).

25 In a particular embodiment, the present invention relates to all members of the herein disclosed family of synapsin III proteins.

The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes an isoform of synapsin III protein;

preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding an isoform synapsin III protein and which has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 4 (SEQ ID NO:2), FIGURE 9 (SEQ ID NO:5), or FIGURE 11 (SEQ ID NO:8).

- 5 The human DNA sequences encoding isoforms of the synapsin III protein, or portions thereof, may be prepared as probes to screen for complementary sequences and genomic clones in the same or alternate species. The present invention extends to probes so prepared that may be provided for screening cDNA and genomic libraries for the presence of an isoform of synapsin III. For example, the probes
- 10 may be prepared with a variety of known vectors, such as the phage λ vector. The present invention also includes the preparation of plasmids including such vectors, and the use of the DNA sequences to construct vectors expressing antisense RNA or ribozymes which would attack the mRNAs of any or all of the DNA sequences set forth in FIGURE 4 (SEQ ID NO:2), FIGURE 9 (SEQ ID NO:5), or FIGURE 11
- 15 (SEQ ID NO:7). Correspondingly, the preparation of antisense RNA and ribozymes are included herein.

The present invention also includes isoforms of synapsin III having the activities noted herein, and that display the amino acid sequences set forth and described above and selected from SEQ ID NO:1, SEQ ID NO:6 or SEQ ID NO:10.

- 20 In a further embodiment of the invention, an isolated nucleic acid molecule encoding an isoform of a synapsin III protein, may be operatively linked to an expression control sequence which may be introduced into an appropriate host. The invention accordingly extends to unicellular hosts transformed with a cloned gene or recombinant DNA molecule comprising a DNA sequence encoding an isoform of
- 25 synapsin III, e.g., synapsin IIIa, synapsin IIIb, or synapsin IIIc, and more particularly, the complete DNA sequence determined from the sequences set forth above and in SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7.

According to other preferred features of certain preferred embodiments of the present invention, a recombinant expression system is provided to produce biologically active animal or human synapsin III isoforms.

The concept of an isoform of synapsin III contemplates that specific factors exist for correspondingly specific interacting protein such as an isoform of synapsin III and the like, as described earlier. Accordingly, the exact structure of each isoform of synapsin III will understandably vary so as to achieve this interacting protein and activity specificity. It is this specificity and the direct involvement of an isoform of synapsin III in the chain of events leading to schizophrenia, that offers the promise of a broad spectrum of diagnostic and therapeutic utilities.

The present invention naturally contemplates several means for preparation of an isoform of synapsin III, including as illustrated herein known recombinant techniques, and the invention is accordingly intended to cover such synthetic preparations within its scope. The isolation of cDNA and amino acid sequences disclosed herein facilitates the reproduction of an isoform of synapsin III by such recombinant techniques, and accordingly, the invention extends to expression vectors prepared from the disclosed DNA sequences for expression in host systems by recombinant DNA techniques, and to the resulting transformed hosts.

The invention includes an assay system for screening of potential drugs effective to modulate activity of isoforms of synapsin III of target mammalian cells by interrupting or potentiating an isoform of synapsin III. In one instance, the test drug could be administered to a cellular sample with the ligand that activates an isoform of synapsin III, or an extract containing an activated isoform of synapsin III, to determine its effect upon the binding activity of an isoform of synapsin III to any chemical sample (including DNA), or to the test drug, by comparison with a control.

The assay system could more importantly be adapted to identify drugs or other entities that are capable of binding to an isoform of synapsin III and/or synapsin III factors or proteins, either in the cytoplasm or in the nucleus, thereby inhibiting or potentiating synaptic activity, and modulating the schizophrenic symptoms in the mammal under treatment. Such assay would be useful in the development of drugs that would be specific against particular cellular activity, or that would potentiate such activity, in time or in level of activity. For example, such drugs might be used to modulate the schizophrenic symptoms, or to treat other pathologicent synapsin are believed to be a factor, as for example, schizophrenia.

- 5
- 10 In yet a further embodiment, the invention contemplates antagonists of the activity of an isoform of a synapsin III. In particular, an agent or molecule that inhibits an isoform of synapsin III. In a specific embodiment, the antagonist can be a peptide having the sequence of a portion of the M domain of a synapsin III.
- 15 The diagnostic utility of the present invention extends to the use an isoform of synapsin III in assays to screen for schizophrenia, and other pathologies wherein deficient neurotransmission is a causative factor.

The present invention likewise extends to the development of antibodies against isoforms of synapsin III, e.g., synapsin IIIa, synapsin IIIb, or synapsin IIIc, including naturally raised and recombinantly prepared antibodies. For example, the antibodies could be used to screen expression libraries to obtain the nucleic acid molecules that encode an isoform of synapsin III. Such antibodies could include both polyclonal and monoclonal antibodies prepared by known genetic techniques, as well as bi-specific (chimeric) antibodies, and antibodies including other functionalities suiting them for additional diagnostic use conjunctive with their capability of modulating synapsis activity.

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In particular, antibodies against specifically phosphorylated factors can be selected and are included within the scope of the present invention for their particular ability in following activated protein. Thus, activity of an isoform of synapsin III or of the specific polypeptides believed to be causally connected thereto may therefore be
5 followed directly by the assay techniques discussed *infra*, through the use of an appropriately labeled quantity of a synapsin III of the invention, or antibodies or analogs thereof.

Thus, isoforms of synapsin III, their analogs and/or derivatives, and any antagonists or antibodies that may be raised thereto, are capable of use in connection with
10 various diagnostic techniques, including immunoassays, such as a radioimmunoassay, using for example, an antibody to an isoform of synapsin III of the invention that has been labeled by either radioactive addition, or radioiodination.

In an immunoassay, a control quantity of the antagonists or antibodies thereto, or
15 the like may be prepared and labeled with an enzyme, a specific binding partner and/or a radioactive element, and may then be introduced into a cellular sample. After the labeled material or its binding partner(s) has had an opportunity to react with sites within the sample, the resulting mass may be examined by known techniques, which may vary with the nature of the label attached.

20 For example, antibodies against specifically phosphorylated factors may be selected and appropriately employed in the exemplary assay protocol, for the purpose of following activated protein as described above.

In the instance where a radioactive label, such as the isotopes ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re are used, known currently available
25 counting procedures may be accomplished by any of the presently utilized

colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques known in the art.

The present invention includes an assay system which may be prepared in the form of a test kit for the quantitative analysis of the extent of the presence of an isoform of synapsin III, or to identify drugs or other agents that may mimic or block the activity of an isoform of synapsin III. The system or test kit may comprise a labeled component prepared by one of the radioactive and/or enzymatic techniques discussed herein, coupling a label to an isoform of synapsin III, agonists and/or antagonists thereof, and one or more additional immunochemical reagents, at least one of which is a free or immobilized ligand, capable either of binding with the labeled component, its binding partner, one of the components to be determined or their binding partner(s).

In a further embodiment, the present invention relates to certain therapeutic methods which would be based upon the activity of an isoform of synapsin III, its subunits, or active fragments thereof, or upon agents or other drugs determined to possess the same activity. A first therapeutic method is associated with the prevention of the manifestations of conditions causally related to or following from the binding activity of an isoform of synapsin III or its subunits, and comprises administering an agent capable of modulating the production and/or activity of an isoform of synapsin III or subunits thereof, either individually or in mixture with each other in an amount effective to prevent the development of those conditions in the host. For example, drugs or other binding partners to an isoform of synapsin III protein may be administered to inhibit or potentiate neurotransmitter activity, as in the treatment of schizophrenia.

Also, the blockade of the action of specific tyrosine phosphatases in the dephosphorylation of activated (phosphorylated) synapsin III proteins, e.g., isoforms of synapsin III, present a method for potentiating the activity of an isoform

of synapsin III protein that would concomitantly potentiate therapies based on synapsin III protein activation.

5 More specifically, the therapeutic method generally referred to herein could include the method for the treatment of various pathologies or other cellular dysfunctions and derangements by the administration of pharmaceutical compositions that may comprise effective inhibitors or enhancers of activation of an isoform of synapsin III such as synapsin IIIa, IIIb, or IIIc, or their subunits, or other equally effective drugs developed for instance by a drug screening assay prepared and used in
10 accordance with a further aspect of the present invention. For example, drugs or other binding partners to an isoform of synapsin III, as represented by SEQ ID NO:1, SEQ ID NO:6 or SEQ ID NO:8, may be administered to inhibit or potentiate neurotransmitter activity.

In particular, the proteins of synapsin III whose sequences are presented in SEQ ID
15 NO:1, SEQ ID NO:6, or SEQ ID NO:8 set forth herein, their antibodies, agonists, antagonists, or active fragments thereof, could be prepared in pharmaceutical formulations for administration in instances wherein modulation of neurotransmission is appropriate, such as to treat various pathologies where such modulation is desirable.

20 In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell
25 Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994)]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D.

Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms shall have the definitions set out
5 below.

The term "synapsin III" generally refers, refer to proteinaceous materials which are isoforms of synapsin III, and extend to those proteins having the amino acid sequence data described herein and presented in FIGURE 4 (SEQ ID NO:1), FIGURE 8 (SEQ ID NO:6), and FIGURE 10 (SEQ ID NO:8), and the profile of
10 activities set forth herein and in the Claims. Accordingly, proteins displaying substantially equivalent or altered activity are likewise contemplated. These modifications may be deliberate, for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such as those obtained through mutations in hosts that are producers of the complex or its named subunits.
15 Also, the terms "synapsin IIIa", "synapsin IIIb", and "synapsin IIIc" are intended to include within their scope proteins specifically recited herein as well as all substantially homologous analogs and allelic variations.

Furthermore, as used herein, the term "isoform" refers to multiple forms of the same protein that differ somewhat in their amino acid sequence. They can be
20 produced by different genes or by alternative splicing of RNA transcripts from the same gene. As set forth herein, synapsin IIIa, IIIb, and IIIc (SEQ ID Nos: 1, 5 and 6 respectively) are isoforms of the synapsin III protein, and are produced from transcription of the synapsin III gene.

25 The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property of immunoglobulin-

binding is retained by the polypeptide. NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J. Biol. Chem.*, **243**:3552-59 (1969), abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

<u>SYMBOL</u>		<u>AMINO ACID</u>	
<u>1-Letter</u>	<u>3-Letter</u>		
	Y	Tyr	tyrosine
10	G	Gly	glycine
	F	Phe	phenylalanine
	M	Met	methionine
	A	Ala	alanine
	S	Ser	serine
15	I	Ile	isoleucine
	L	Leu	leucine
	T	Thr	threonine
	V	Val	valine
	P	Pro	proline
20	K	Lys	lysine
	H	His	histidine
	Q	Gln	glutamine
	E	Glu	glutamic acid
	W	Trp	tryptophan
25	R	Arg	arginine
	D	Asp	aspartic acid
	N	Asn	asparagine
	C	Cys	cysteine

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues. The above Table is presented to correlate the three-letter and one-letter notations which may appear alternately herein.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

- 10 Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

- 25 An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell

when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

The term "oligonucleotide," as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For
5 example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand to hybridize therewith and thereby
10 form the template for the synthesis of the extension product.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA
15 has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA
20 has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone
25 of a primary cell that is capable of stable growth *in vitro* for many generations.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the

nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

It should be appreciated that also within the scope of the present invention are isolated nucleic acid molecules which encode isoforms of synapsin III having an amino acid sequence of SEQ ID NO:1, SEQ ID NO:6, and SEQ ID NO:8, but which are degenerate to SEQ ID NO:2, SEQ ID NO:5, and SEQ ID NO:7. By "degenerate to" is meant that a different three-letter codon is used to specify a particular amino acid. It is well known in the art that the following codons can be used interchangeably to code for each specific amino acid:

15	Phenylalanine (Phe or F)	UUU or UUC
	Leucine (Leu or L)	UUA or UUG or CUU or CUC or CUA or CUG
	Isoleucine (Ile or I)	AUU or AUC or AUA
	Methionine (Met or M)	AUG
	Valine (Val or V)	GUU or GUC or GUA or GUG
20	Serine (Ser or S)	UCU or UCC or UCA or UCG or AGU or AGC
	Proline (Pro or P)	CCU or CCC or CCA or CCG
	Threonine (Thr or T)	ACU or ACC or ACA or ACG
	Alanine (Ala or A)	GCU or GCG or GCA or GCC
	Tyrosine (Tyr or Y)	UAU or UAC
25	Histidine (His or H)	CAU or CAC
	Glutamine (Gln or Q)	CAA or CAG
	Asparagine (Asn or N)	AAU or AAC
	Lysine (Lys or K)	AAA or AAG

Aspartic Acid (Asp or D)	GAU or GAC
Glutamic Acid (Glu or E)	GAA or GAG
Cysteine (Cys or C)	UGU or UGC
Arginine (Arg or R)	CGU or CGC or CGA or CGG or AGA or AGG
5 Glycine (Gly or G)	GGU or GGC or GGA or GGG
Tryptophan (Trp or W)	UGG
Termination codon	UAA (ochre) or UAG (amber) or UGA (opal)

It should be understood that the codons specified above are for RNA sequences.

- 10 The corresponding codons for DNA have a T substituted for U.

Mutations can be made in SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7 such that a particular codon is changed to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid

15 in the resulting protein in a non-conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid

20 belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or

25 binding characteristics of the resulting protein.

The following is one example of various groupings of amino acids:

Amino acids with nonpolar R groups

Alanine

Valine

Leucine

Isoleucine

5 Proline

Phenylalanine

Tryptophan

Methionine

Amino acids with uncharged polar R groups

10 Glycine

Serine

Threonine

Cysteine

Tyrosine

15 Asparagine

Glutamine

Amino acids with charged polar R groups (negatively charged at Ph 6.0)

Aspartic acid

Glutamic acid

20 Basic amino acids (positively charged at pH 6.0)

Lysine

Arginine

Histidine (at pH 6.0)

Another grouping may be those amino acids with phenyl groups:

Phenylalanine

Tryptophan

Tyrosine

5 Another grouping may be according to molecular weight (i.e., size of R groups):

	Glycine	75
	Alanine	89
	Serine	105
	Proline	115
10	Valine	117
	Threonine	119
	Cysteine	121
	Leucine	131
	Isoleucine	131
15	Asparagine	132
	Aspartic acid	133
	Glutamine	146
	Lysine	146
	Glutamic acid	147
20	Methionine	149
	Histidine (at pH 6.0)	155
	Phenylalanine	165
	Arginine	174
	Tyrosine	181
25	Tryptophan	204

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH₂ can be maintained.

5 Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its
10 particularly planar structure, which induces β -turns in the protein's structure.

Two amino acid sequences are "substantially homologous" when at least about 70% of the amino acid residues (preferably at least about 80%, and most preferably at least about 90 or 95%) are identical, or represent conservative substitutions.

A "heterologous" region of a DNA construct is an identifiable segment of DNA
15 within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not
20 found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof,
25 that binds a specific epitope. The term encompasses polyclonal, monoclonal, and

chimeric antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that
5 specifically binds antigen.

The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially
10 intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', F(ab')₂ and F(v), which portions are preferred for use in the therapeutic methods described herein.

Fab and F(ab')₂ portions of antibody molecules are prepared by the proteolytic
15 reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from F(ab')₂ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and
20 followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of
25 immunoreacting with a particular antigen. A monoclonal antibody thus typically

displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

- 5 The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

The phrase "therapeutically effective amount" is used herein to mean an amount
10 sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in symptoms related to schizophrenia, such as hallucinations, delusions, bizarre behavior, thought disorder, lack of motivation, social withdrawal and apathy.

- 15 A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the
20 DNA sequence under the control of the expression control ion of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as
25 a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate

conditions of temperature and solution ionic strength (*see* Sambrook et al., *supra*). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55°, can be used, *e.g.*, 5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m , *e.g.*, 40% formamide, with 5x or 6x SSC. High stringency hybridization conditions correspond to the highest T_m , *e.g.*, 50% formamide, 5x or 6x SSC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (*see* Sambrook et al., *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (*see* Sambrook et al., *supra*, 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid is at least about 10 nucleotides; preferably at least about 15 nucleotides; and more preferably the length is at least about 20 nucleotides; and most preferably 30 nucleotides.

25 In a specific embodiment, the term "standard hybridization conditions" refers to a T_m of 55°C, and utilizes conditions as set forth above. In a preferred embodiment, the T_m is 60°C; in a more preferred embodiment, the T_m is 65°C. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid

molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (*see* Sambrook et al., *supra*). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55°, can be used, *e.g.*, 5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m , *e.g.*, 40% formamide, with 5x or 6x SSC. High stringency hybridization conditions correspond to the highest T_m , *e.g.*, 50% formamide, 5x or 6x SSC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (*see* Sambrook et al., *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (*see* Sambrook et al., *supra*, 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid is at least about 10 nucleotides; preferably at least about 15 nucleotides; and more preferably the length is at least about 30 nucleotides; and most preferably 40 nucleotides.

In a specific embodiment, the term "standard hybridization conditions" refers to a T_m of 55°C, and utilizes conditions as set forth above. In a preferred embodiment, the T_m is 60°C; in a more preferred embodiment, the T_m is 65°C.

In its primary aspect, the present invention concerns the identification of an isoform of synapsin III, e.g., synapsin IIIa, synapsin IIIb, and synapsin IIIc.

As stated above, the present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes an isoform of synapsin III, or a fragment thereof, comprising an amino acid sequence set forth in FIGURE 4 (SEQ ID NO:1), FIGURE 8 (SEQ ID NO:5) or FIGURE 10 (SEQ ID NO:8); preferably an isolated nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding an isoform of synapsin III having, wherein the nucleic acid molecule comprises a DNA sequence a nucleotide sequence or is complementary to a DNA sequence of the invention shown in FIGURE 4 (SEQ ID NO:2), FIGURE 9 (SEQ ID NO:5), or FIGURE 11 (SEQ ID NO:7).

The possibilities both diagnostic and therapeutic that are raised by the existence of an isoform of synapsin III, derive from the fact that factors of isoforms of synapsin III appear to participate in direct and causal protein-protein interaction between isoforms of synapsin III, and those factors that thereafter affect neurotransmission. As suggested earlier and elaborated further on herein, the present invention contemplates pharmaceutical intervention in the cascade of reactions in which an isoform of synapsin III is implicated, to modulate the activity initiated by an isoform of synapsin III.

Thus, in instances where it is desired to reduce or inhibit the resulting from a particular stimulus or factor, an appropriate inhibitor of an isoform of synapsin III, e.g., an inhibitor of synapsin IIIa, synapsin IIIb, or synapsin IIIc, could be introduced to block the interaction of an isoform of synapsin III with those factors causally connected with synapsin III. Correspondingly, instances where insufficient neurotransmission is taking place could be remedied by the introduction of additional quantities of an isoform of synapsin III or its chemical or pharmaceutical cognates, analogs, fragments and the like.

As discussed earlier, isoforms of synapsin III, their binding partners or other ligands or agents exhibiting either mimicry or antagonism thereto or control over their production, may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient experiencing an adverse medical condition associated with an isoform of synapsin III for the treatment thereof. A variety of administrative techniques may be utilized, among them parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections, catheterizations and the like. Average quantities of an isoform of synapsin III or its subunits may vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

Also, antibodies including both polyclonal and monoclonal antibodies, and drugs that modulate the production or activity of an isoform of a synapsin III and/or its subunits may possess certain diagnostic applications and may for example, be utilized for the purpose of detecting and/or measuring conditions such as viral infection or the like. For example, an isoform of synapsin III or its subunits may be used to produce both polyclonal and monoclonal antibodies to themselves in a variety of cellular media, by known techniques such as the hybridoma technique utilizing, for example, fused mouse spleen lymphocytes and myeloma cells. Likewise, small molecules that mimic or antagonize the activity(ies) of an isoform of synapsin III of the invention may be discovered or synthesized, and may be used in diagnostic and/or therapeutic protocols.

The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al., "Hybridoma Techniques" (1980); Hammerling et al., "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett et al., "Monoclonal Antibodies" (1980); see

also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890.

Panels of monoclonal antibodies produced against peptides of isoforms of synapsin III can be screened for various properties; i.e., isotope, epitope, affinity, etc. Of particular interest are monoclonal antibodies that neutralize the activity of an isoform of synapsin III or its subunits. Such monoclonals can be readily identified in synapsin III activity assays. High affinity antibodies are also useful when immunoaffinity purification of a native or recombinant isoform of synapsin III is possible.

10 Preferably, an anti-synapsin III antibody, i.e., an antibody made against an isoform of synapsin III, used in the diagnostic methods of this invention is an affinity purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for an anti-synapsin III antibody molecule, e.g., an antibody made against an isoform of synapsin III, used herein be
15 in the form of Fab, Fab', F(ab')₂ or F(v) portions of whole antibody molecules.

As suggested earlier, the diagnostic method of the present invention comprises examining a cellular sample or medium by means of an assay including an effective amount of an antagonist to an isoform of a synapsin III/protein, such as an anti-synapsin III antibody, preferably an affinity-purified polyclonal antibody, and more
20 preferably a mAb. As previously discussed, patients capable of benefiting from this method include those suffering from hallucinations, delusions, bizarre behavior and thought disorder, as well as negative symptoms such as lack of motivation, social withdrawal and apathy. Methods for isolating the synapsin III and inducing anti-synapsin III antibodies and for determining and optimizing the ability of anti-
25 synapsin III antibodies to assist in the examination of the target cells are all well-known in the art.

Methods for producing polyclonal anti-polypeptide antibodies are well-known in the art. See U.S. Patent No. 4,493,795 to Nestor et al. A monoclonal antibody, typically containing Fab and/or F(ab')₂ portions of useful antibody molecules, can be prepared using the hybridoma technology described in *Antibodies - A Laboratory*
5 *Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory, New York (1988), which is incorporated herein by reference in its entirety. Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized with the binding portion of an
10 isoform of synapsin III or an origin-specific DNA-binding portion thereof.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact with an isoform of synapsin III and their ability to
15 inhibit specified synapsin III activity in target cells.

A monoclonal antibody useful in practicing the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period
20 sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known techniques.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media, inbred mice and
25 the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., *Virology* **8**:396 (1959)) supplemented with 4.5 gm/l

glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Methods for producing monoclonal antibodies against isoforms of synapsin III are also well-known in the art. See Niman et al., *Proc. Natl. Acad. Sci. USA*, **80**:4949-
5 4953 (1983). Typically, an isoform of synapsin III or a peptide analog is used either alone or conjugated to an immunogenic carrier, as the immunogen in the before described procedure for producing anti-synapsin III monoclonal antibodies. The hybridomas are screened for the ability to produce an antibody that immunoreacts with an isoform of a synapsin III, or a peptide analog or fragment of
10 an isoform of a synapsin III.

The present invention further contemplates therapeutic compositions useful in practicing the therapeutic methods of this invention. A subject therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more isoforms of a synapsin III, polypeptide analog thereof or
15 fragment thereof, as described herein as an active ingredient. In a preferred embodiment, the composition comprises an antigen capable of modulating the specific binding of an isoform of synapsin III within a target cell.

The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such
20 compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for
25 example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of

auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

A polypeptide, analog or active fragment can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms.

- 5 Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for
- 10 example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit dose, for

15 example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

- 20 The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition or neutralization of synapsin III binding capacity desired. Precise amounts of active ingredient required
- 25 to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of

active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

Exemplary formulations are given below:

Formulations

Intravenous Formulation I

10	<u>Ingredient</u>	<u>mg/ml</u>
	isoform of synapsin III	10.0
	dextrose USP	45.0
	sodium bisulfite USP	3.2
	edetate disodium USP	0.1
15	water for injection q.s.a.d.	1.0 ml

Intravenous Formulation II

	<u>Ingredient</u>	<u>mg/ml</u>
	analog of synapsin III	10.0
	sodium bisulfite USP	3.2
20	disodium edetate USP	0.1
	water for injection q.s.a.d.	1.0 ml

Intravenous Formulation III

	<u>Ingredient</u>	<u>mg/ml</u>
	analog of synapsin III	10.0
25	sodium bisulfite USP	3.2

disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation IV

<u>Ingredient</u>	<u>mg/ml</u>
5 analog of synapsin III	10.0
dextrose USP	45.0
sodium bisulfite USP	3.2
edetate disodium USP	0.1
water for injection q.s.a.d.	1.0 ml

10 Intravenous Formulation V

<u>Ingredient</u>	<u>mg/ml</u>
antagonist of an isoform of synapsin III	5.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
15 water for injection q.s.a.d.	1.0 ml

As used herein, "pg" means picogram, "ng" means nanogram, "ug" or " μg " mean microgram, "mg" means milligram, "ul" or " μl " mean microliter, "ml" means milliliter, "l" means liter.

Another feature of this invention is the expression of the DNA sequences disclosed
 20 herein. As is well known in the art, DNA sequences may be expressed by
 operatively linking them to an expression control sequence in an appropriate
 expression vector and employing that expression vector to transform an appropriate
 unicellular host.

Such operative linking of a DNA sequence of this invention to an expression control
 25 sequence, of course, includes, if not already part of the DNA sequence, the

provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence.

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, *e.g.*, *E. coli* plasmids col E1, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAs, *e.g.*, the numerous derivatives of phage λ , *e.g.*, NM989, and other phage DNA, *e.g.*, M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like. Especially useful is the expression vector which is the plasmid pET15b (Novagen, Madison, WI).

Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the *lac* system, the *trp* system, the *TAC* systator and promoter regions of phage λ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (*e.g.*, Pho5), the promoters of the yeast α -mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and

prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, R1.1, B-W and L-M cells, African Green Monkey kidney cells (*e.g.*, COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (*e.g.*, Sf9), and human cells and plant cells in tissue culture.

- 5 It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired
- 10 expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.
- 15 In selecting an expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be selected by consideration of, *e.g.*, their compatibility with
- 20 the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host to be expressed, and the ease of purification of the expression products.

Considering these and other factors a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will

25 express the DNA sequences of this invention on fermentation or in large scale animal culture.

It is further intended that analogs of isoforms of synapsin III may be prepared from nucleotide sequences of the protein complex/subunit derived within the scope of the present invention. Analogs, such as fragments, may be produced, for example, by pepsin digestion of synapsin III material. Other analogs, such as muteins, can be produced by standard site-directed mutagenesis of nucleic acids encoding isoforms of synapsin III, such as an isolated nucleic acid of FIGURE 4 (SEQ ID NO:2), FIGURE 9 (SEQ ID NO:5), or FIGURE 11 (SEQ ID NO:7). Analogs exhibiting "synapsin III activity" such as small molecules, whether functioning as promoters or inhibitors, may be identified by known *in vivo* and/or *in vitro* assays.

10 As mentioned above, an isolated nucleic acid molecule encoding an isoform of synapsin III can be prepared synthetically rather than cloned. The molecule can be designed with the appropriate codons for an isoform of the synapsin III. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping
15 oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, *e.g.*, Edge, *Nature*, **292**:756 (1981); Nambair et al., *Science*, **223**:1299 (1984); Jay et al., *J. Biol. Chem.*, **259**:6311 (1984).

Synthetic nucleic acid molecules allow convenient construction of genes which will express analogs or "muteins" of isoforms of synapsin III. Alternatively, DNA
20 encoding muteins can be made by site-directed mutagenesis of native synapsin III genes or cDNAs, and muteins can be made directly using conventional polypeptide synthesis.

A general method for site-specific incorporation of unnatural amino acids into proteins is described in Christopher J. Noren, Spencer J. Anthony-Cahill, Michael
25 C. Griffith, Peter G. Schultz, *Science*, **244**:182-188 (April 1989). This method may be used to create analogs with unnatural amino acids.

The present invention further extends to the preparation of antisense oligonucleotides and ribozymes that may be used to interfere with the expression of an isolated nucleic acid molecule encoding an isoform of synapsin III at the translational level. This approach utilizes antisense nucleic acid and ribozymes to
5 block translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. (See Weintraub, 1990; Marcus-Sekura, 1988.) In the cell, they hybridize to that mRNA, forming a double
10 stranded molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation codon will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when
15 introducing them into cells producing isoforms of synapsin III. Antisense methods have been used to inhibit the expression of many genes *in vitro* (Marcus-Sekura, 1988; Hambor et al., 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single stranded RNA molecules in a manner somewhat analogous to DNA
20 restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs have the ability to excise their own introns. By modifying the nucleotide sequence of these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988.). Because they are sequence-specific, only mRNAs with
25 particular sequences are inactivated.

Investigators have identified two types of ribozymes, *Tetrahymena*-type and "hammerhead"-type. (Hasselhoff and Gerlach, 1988) *Tetrahymena*-type ribozymes

recognize four-base sequences, while "hammerhead"-type recognize eleven- to
eighteen-base sequences. The longer the recognition sequence, the more likely it is
to occur exclusively in the target mRNA species. Therefore, hammerhead-type
ribozymes are preferable to *Tetrahymena*-type ribozymes for inactivating a specific
5 mRNA species, and eighteen base recognition sequences are preferable to shorter
recognition sequences.

The DNA sequences described herein may thus be used to prepare antisense
molecules against, and ribozymes that cleave mRNAs encoding isoforms of
synapsin III, and their ligands.

10 The present invention also relates to a variety of diagnostic applications, including
methods for detecting the presence of stimuli such as the earlier referenced
interacting proteins by reference to their ability to elicit the activities which are
mediated by an isoform of synapsin III. As mentioned earlier, isoforms of synapsin
III can be used to produce antibodies to themselves by a variety of known
15 techniques, and such antibodies could then be isolated and utilized as in tests for the
presence of particular synapsin III activity in suspect target cells.

As described in detail above, antibody(ies) to an isoform of synapsin III can be
produced and isolated by standard methods including the well known hybridoma
techniques. For convenience, the antibody(ies) to an isoform of synapsin III will be
20 referred to herein as Ab₁ and antibody(ies) raised in another species as Ab₂.

The presence of an isoform of synapsin III in cells can be ascertained by the usual
immunological procedures applicable to such determinations. A number of useful
procedures are known. Three such procedures which are especially useful utilize
either an isoform of synapsin III labeled with a detectable label, antibody Ab₁
25 labeled with a detectable label, or antibody Ab₂ labeled with a detectable label. The
procedures may be summarized by the following equations wherein the asterisk

indicates that the particle is labeled, and "synIII" stands for an isoform of synapsin III:

- A. $\text{synIII}^* + \text{Ab}_1 = \text{synIII}^*\text{Ab}_1$
- B. $\text{synIII} + \text{Ab}^* = \text{synIIIAb}_1^*$
- 5 C. $\text{synIII} + \text{Ab}_1 + \text{Ab}_2^* = \text{synIIIAb}_1\text{Ab}_2^*$

The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. The "competitive" procedure, Procedure A, is described in U.S. Patent Nos. 3,654,090 and 3,850,752. Procedure C, the "sandwich" procedure, is described in U.S.

- 10 Patent Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the "double antibody," or "DASP" procedure.

In each instance, an isoform of synapsin III forms complexes with one or more antibody(ies) or binding partners and one member of the complex is labeled with a detectable label. The fact that a complex has formed and, if desired, the amount
15 thereof, can be determined by known methods applicable to the detection of labels.

It will be seen from the above, that a characteristic property of Ab_2 is that it will react with Ab_1 . This is because Ab_1 raised in one mammalian species has been used in another species as an antigen to raise the antibody Ab_2 . For example, Ab_2 may be raised in goats using rabbit antibodies as antigens. Ab_2 therefore would be
20 anti-rabbit antibody raised in goats. For purposes of this description and claims, Ab_1 will be referred to as a primary or anti-synapsin III antibody, and Ab_2 will be referred to as a secondary or anti- Ab_1 antibody.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

- 5 An isoform of synapsin III or its binding partner(s) can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re .

- Enzyme labels are likewise useful, and can be detected by any of the presently
10 utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, β -glucuronidase,
15 β -D-glucosidase, β -D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

- A particular assay system developed and utilized in accordance with the present
20 invention, is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the labeled and unlabeled material after which binding studies are conducted to determine the extent to which the labeled material binds to the be ascertained.

- 25 Accordingly, a purified quantity of an isoform of synapsin III, e.g., synapsin IIIa, synapsin IIIb or synapsin IIIc may be radiolabeled and combined, for example, with

antibodies or other inhibitors thereto, after which binding studies would be carried out. Solutions would then be prepared that contain various quantities of labeled and unlabeled uncombined synapsin III, and cell samples would then be inoculated and thereafter incubated. The resulting cell monolayers are then washed, solubilized
5 and then counted in a gamma counter for a length of time sufficient to yield a standard error of <5%. These data are then subjected to Scatchard analysis after which observations and conclusions regarding material activity can be drawn.

While the foregoing is exemplary, it illustrates the manner in which a receptor assay may be performed and utilized, in the instance where the cellular binding ability of
10 the assayed material may serve as a distinguishing characteristic.

Another assay useful and contemplated in accordance with the present invention is known as a "cis/trans" assay. Briefly, this assay employs two genetic constructs, one of which is typically a plasmid that continually expresses a particular receptor of interest when transfected into an appropriate cell line, and the second of which is
15 a plasmid that expresses a reporter such as luciferase, under the control of a receptor/ligand complex. Thus, for example, if it is desired to evaluate a compound as a ligand for a particular receptor, one of the plasmids would be a construct that results in expression of the receptor in the chosen cell line, while the second plasmid would possess a promoter linked to the luciferase gene in which the
20 response element to the particular receptor is inserted. If the compound under test is an agonist for the receptor, the ligand will complex with the receptor, and the resulting complex will bind the response element and initiate transcription of the luciferase gene. The resulting chemiluminescence is then measured photometrically, and dose response curves compared to those of known ligands.
25 The foregoing protocol is described in detail in U.S. Patent No. 4,981,784 and PCT International Publication No. WO 88/03168, for which purpose the artisan is referred.

In a further embodiment of this invention, commercial test kits suitable for use by a medical professional may be prepared to determine the presence or absence of predetermined synapsin III activity or predetermined synapsin III activity capability in suspected target cells. In accordance with the testing techniques discussed above, one class of such kits will contain at least a labeled isoform of synapsin III or its binding partner, for instance an antibody specific thereto, and directions, of course, depending upon the method selected, e.g., "competitive," "sandwich," "DASP" and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

- 10 Accordingly, a test kit may be prepared for the demonstration of the presence or capability of cells for predetermined synapsin III activity, comprising:
- (a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of a factor of an isoform of synapsin III or a specific binding partner thereto, to a detectable label;
 - 15 (b) other reagents; and
 - C) directions for use of said kit.

More specifically, the diagnostic test kit may comprise:

- (a) a known amount of an isoform of synapsin III as described above (or a binding partner) generally bound to a solid phase to form an immunosorbent, or in 20 the alternative, bound to a suitable tag, or plural such end products, etc. (or their binding partners) one of each;
- (b) if necessary, other reagents; and
- (c) directions for use of said test kit.

In a further variation, the test kit may be prepared and used for the purposes stated 25 above, which operates according to a predetermined protocol (e.g. "competitive," "sandwich," "double antibody," etc.), and comprises:

- (a) a labeled component which has been obtained by coupling an isoform of synapsin III to a detectable label;
- (b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand is selected from the group consisting of:
- 5 (I) a ligand capable of binding with the labeled component (a);
- (ii) a ligand capable of binding with a binding partner of the labeled component (a);
- (iii) a ligand capable of binding with at least one of the component(s) to
- 10 be determined; and
- (iv) a ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; and
- (c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between
- 15 the synapsin III and a specific binding partner thereto.

The present invention also includes a test kit for genetic screening which can be utilized to identify mutations an isoform of synapsin III or its encoding cDNA. By identifying patients with mutated synapsin III DNA and comparing the mutation to a base developed by correlating a particular mutation in an isoform of synapsin III

20 with a particular neuropsychiatric state or disease, identification and/or confirmation of, a particular neuropsychiatric state or disease can be made. Accordingly, such a kit would comprise a PCR-based test that would involve transcribing the patients mRNA with a specific primer, and amplifying the resulting cDNA using another set of primers. The amplified product would be detectable by

25 gel electrophoresis and could be compared with known standards for the various isoforms of synapsin III. Accordingly, such a kit would utilize a patient's blood or serum sample and the DNA would be extracted using standard techniques. Primers flanking a known mutation are then used to amplify a piece of the synapsin III gene.

The amplified piece would then be sequenced to determine the presence of a mutation.

In accordance with the above, an assay system for screening potential drugs effective to modulate the activity of an isoform of synapsin III may be prepared. A particular isoform synapsin III may be introduced into a test system, and the prospective drug may also be introduced into the resulting cell culture, and the culture thereafter examined to observe any changes in activity of an isoform of synapsin III in the cells, due either to the addition of the prospective drug alone, or due to the effect of added quantities of an isoform of synapsin III.

10

PRELIMINARY CONSIDERATIONS

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE 1

15 Experimental Details

Searching for Exons in Chromosome 22

Homology to a highly conserved exon of synapsin I and II, exon 9, was observed in a small region of chromosome 22, known as H5770. This region of chromosome 22 has been studied intensively because of its possible association with human disease, and is currently being sequenced as part of the human genome project. Cosmid sequences flanking the initial observed region of homology were identified and searched for other homologous exons. Eventually, 9 out of 13 exons of a new synapsin were discovered in this region of chromosome 22 (FIGURE 1). This

region of chromosome 22 was previously identified as one that would likely contain a susceptibility gene for schizophrenia (9-17) (FIGURE 2 and Table 1, below).

TABLE 1
Reported Significance of Linkage Among Various Studies

<u>D22S</u> <u>Marker</u>	Coon et al, 1994	Polymeropoulos et al, 1994	Vallada et al, 1995a	Vallada et al, 1995b	Kalsi et al., 1995	Moises et al. 1995*	Lasseter et al., 1995	Schwab et al., 1995	Gill et al, 1996
264			0.14				0.015		
315	0.299	ns							
268			0.46	0.017			0.105		
304								0.068	
281	0.656	0.026							
278	0.312	0.019	0.01	0.008		0.02	0.003		0.001
283	0.730	0.017	0.001	0.005	0.696		0.013		
IL2R β	0.557	ns	0.43				0.046	0.382	
279	0.20	0.042							
276	0.016	ns							
CYP2D	0.221		0.11						
270	0.118	ns							
307			0.71						
282			0.05				0.162		
274	0.043	ns	0.64		0.831				

ns = not significant

If blank, D22S marker was not analyzed.

* = TDT analysis (transmission disequilibrium test). All other figures are reported significance of sib pair analyses.

The existence of homologous synapsin sequences in genomic DNA, however, does not necessarily mean that it is functional. Nonfunctional genes do exist in the human genome and are termed pseudogenes. To demonstrate that the new synapsin sequence was transcribed and expressed, it was demonstrated that spliced, mature
5 RNA existed. It was further assumed, as with like other synapsins, that this new synapsin was expressed in brain.

Poly A+ human brain RNA was obtained from Clontech, and the RNA was reverse transcribed using random primers (23). The resulting cDNA served as a template for PCR using various primer pairs spanning different sets of exons. As
10 depicted in FIGURE 3, three PCR products were generated spanning the entire coding region of synapsin III. The sequence deduced from these PCR products is consistent with the existence of transcribed, spliced mature mRNA corresponding to synapsin III in human brain.

This confirms that the synapsin III gene is not a pseudogene, but a functional gene
15 which gives rise to a protein in the brain.

Sequence of Synapsin III

Sequencing of the synapsin III gene revealed a novel DNA molecule encoding isoforms of the human synapsin III protein, e.g., synapsin IIIa, IIIb and IIIc. Synapsin IIIa is homologous to the known DNA sequences which encode the human
20 synapsin I and synapsin II protein. One region of synapsin III, known as domain M, bears no homology to the other synapsins, and no homology to other proteins in Genbank.

The nucleotide sequence (upper sequence) and deduced amino acid sequence (lower sequence) of human synapsin IIIa is depicted in FIGURE 4. The protein sequence
25 of synapsin IIIa is compared to synapsins IIa and Ia in FIGURE 5.

EXAMPLE 2

Northern blot analysis demonstrating tissue specific distribution and brain localization. (This experiment has already been performed, and synapsin IIIa is expressed predominantly in the brain. The figure is pending.)

5

EXAMPLE 3

Premade human northern blots from Clontech were hybridized to a probe derived from the 3' UTR of human synapsin III. Hybridization and wash conditions were performed according to the manufacturer's specification.

EXAMPLE 4

10 Example 3 demonstrates that the antibody G304, which recognizes domain E of all synapsins, can recognize synapsins Ia, IIa, and IIIa migrate together.

Various mouse brain regions were dissected, homogenized, and boiled in 1% SDS. 100 μ g of protein were electrophoresed on 7.5% SDS polyacrylamide gels, and electroblotted onto a nitrocellulose filter. The resulting western blot was then
15 incubated with anti-synapsin antibody (G304; 1:5000 dilution) which detects all three synapsins, followed by incubation with alkaline phosphatase-conjugated goat anti-rabbit antibody. The blot was developed using the alkaline phosphatase substrates NBT and BCIP.

While the invention has been described and illustrated herein by references to
20 various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art.

The following is a list of documents related to the above disclosure and particularly to the experimental procedures and discussions. The documents should be considered as incorporated by reference in their entirety.

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WHAT IS CLAIMED IS:

- 1 1. An isoform of synapsin III, comprising a material selected from the group
2 consisting of a protein, active fragments thereof, agonists thereof, mimics
3 thereof, and combinations thereof, said isoform having the following
4 characteristics:
 - 5 a) it is localized to neurons and nerve terminals; and
 - 6 b) it is associated with synaptic vesicles.

- 1 2. The isoform of Claim 1 which is a member of the synapsin III family of
2 synapsin III, and comprises synapsin IIIa, synapsin IIIb, or synapsin IIIc.

- 1 3. The isoform of Claim 1 which is cytoplasmic in origin.

- 1 4. The isoform of Claim 1 which is a polypeptide comprising an amino acid
2 sequence of SEQ ID NO:1, SEQ ID NO:6, or SEQ ID NO:8.

- 1 5. The isoform of Claim 1 which is derived from human cells.

- 1 6. The isoform of Claim 1 labeled with a detectable label.

- 1 7. The isoform of Claim 6 wherein the label is selected from enzymes,
2 chemicals which fluoresce and radioactive elements.

- 1 8. An antibody to an isoform of synapsin III having the following
2 characteristics:
 - 3 a) it is localized to neurons and nerve terminals; and
 - 4 b) it is associated with synaptic vesicles.

- 1 9. The antibody of Claim 8 which is a polyclonal antibody.

- 1 10. The antibody of Claim 8 which is a monoclonal antibody.
- 1 11. An immortal cell line that produces a monoclonal antibody according to
2 Claim 10.
- 1 12. The antibody of Claim 8 labeled with a detectable label.
- 1 13. The antibody of Claim 12 wherein the label is selected from enzymes,
2 chemicals which fluoresce and radioactive elements.
- 1 14. An isolated nucleic acid molecule, or or degenerate variant thereof, which
2 encodes a synapsin III, or a fragment thereof, wherein said isolated nucleic acid
3 molecule comprises a DNA sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ
4 ID NO:7), degenerate variants of said sequences, or fragments thereof.
- 1 15. A recombinant DNA molecule comprising a DNA sequence or degenerate
2 variant thereof, which encodes an isoform of synapsin III, or a fragment thereof,
3 selected from the group consisting of:
4 (A) the DNA sequence of FIGURE 4 (SEQ ID NO:2), FIGURE 9
5 (SEQ ID NO:5), FIGURE 11 (SEQ ID NO:7);
6 (B) DNA sequences that hybridize to any of the foregoing DNA
7 sequences under standard hybridization conditions; and
8 (c) DNA sequences that code on expression for an amino acid
9 sequence encoded by any of the foregoing DNA sequences.
- 1 16. The recombinant DNA molecule of either of Claims 14 or 15, wherein
2 said DNA sequence is operatively linked to an expression control sequence.

1 17. The recombinant DNA molecule of Claim 16, wherein said expression
2 control sequence is selected from the group consisting of the early promoters of
3 SV40, late promoters of SV40 of SV40, promoters of adenovirus, the *lac*
4 system, the *trp* system, the *TAC* system, the *TRC* system, the major operator and
5 promoter regions of phage λ , the control regions of fd coat protein, the promoter
6 for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the
7 promoters of the yeast α -mating factors.

1 18. A probe capable of screening for the synapsin III in alternate species
2 prepared from the isolated nucleic acid molecule of Claim 14.

1 19. A unicellular host transformed with a recombinant DNA molecule
2 comprising a DNA sequence or degenerate variant thereof, which encodes a
3 synapsin III, or a fragment thereof, selected from the group consisting of:

4 (A) the DNA sequence of FIGURE 4 (SEQ ID NO:2), FIGURE 9
5 (SEQ ID NO:5), or FIGURE 11 (SEQ ID NO:7);

6 (B) DNA sequences that hybridize to any of the foregoing DNA
7 sequences under standard hybridization conditions; and

8 (c) DNA sequences that code on expression for an amino acid
9 sequence encoded by any of the foregoing DNA sequences;

10 wherein said DNA sequence is operatively linked to an expression control
11 sequence.

1 20. The unicellular host of Claim 19 wherein the unicellular host is selected
2 from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*,
3 yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10
4 cells, plant cells, insect cells, and human cells in tissue culture.

1 21. A method for detecting the presence or activity of an isoform of synapsin
2 III, said isoform having the following characteristics:

3 a) it is localized to neurons and nerve terminals; and
4 b) it is associated with synaptic vesicles;
5 wherein said isoform is measured by:
6 A. contacting a biological sample from a mammal in which
7 the presence or activity of said isoform is suspected with a binding partner of
8 said isoform under conditions that allow binding of said isoform to said binding
9 partner to occur; and
10 B. detecting whether binding has occurred between said
11 isoform from said sample and the binding partner;
12 wherein the detection of binding indicates that presence or activity of said
13 synapsin III in said sample.

1 22. A method for detecting the presence and activity of an isoform of
2 synapsin III in mammals comprising detecting the presence or activity of an
3 isoform of synapsin III according to the method of Claim 21, wherein detection
4 of the presence or activity of the isoform indicates the presence or absence of a
5 deficiency or abnormality in neurotransmission.

1 23. The method of Claim 22 wherein said deficiency or abnormality results in
2 a susceptibility to schizophrenia.

1 24. The method of Claim 22 wherein said detection results in a diagnosis of
2 schizophrenia.

1 25. A method for detecting the binding sites for said isoform of synapsin III
2 having the following characteristics:
3 a) it is localized to neurons and nerve terminals; and
4 b) it is associated with synaptic vesicles,

- 5 A. placing a labeled isoform of synapsin III sample in contact
6 with a biological sample from a mammal in which binding sites for said isoform
7 are suspected;
- 8 B. examining said biological sample in binding studies for the
9 presence of said labeled isoform;
- 10 wherein the presence of said labeled isoform indicates a binding site for a
11 synapsin III.

- 1 26. A method of testing the ability of a drug or other entity to modulate the
2 activity of an isoform of synapsin III which comprises
- 3 A. culturing a colony of test cells which has a receptor for the
4 isoform in a growth medium containing the isoform;
- 5 B. adding the drug under test; and
- 6 C. measuring the reactivity of the isoform with the receptor on said
7 colony of test cells,
8 wherein said isoform has the following characteristics:
- 9 a) it is localized to neurons and nerve terminals;
- 10 b) it is associated with synaptic vesicles; and
- 11 c) it possess an apparent molecular weight of 63,000 daltons.

- 1 27. An assay system for screening drugs and other agents for ability to
2 modulate the production of an isoform of synapsin III, comprising:
- 3 A. culturing an observable cellular test colony inoculated with a drug
4 or agent;
- 5 B. harvesting a supernatant from said cellular test colony; and
- 6 C. examining said supernatant for the presence of said isoform,
7 wherein an increase or a decrease in a level of said isoform indicates the ability
8 of a drug to modulate the activity of said said isoform, said isoform having the
9 following characteristics:
- 10 a) it is localized to neurons and nerve terminals; and

11 b) it is associated with synaptic vesicles.

1 28. A test kit for the demonstration of an isoform of synapsin III in a
2 eukaryotic cellular sample, comprising:
3 A. a predetermined amount of a detectably labelled specific
4 binding partner of an isoform of synapsin III, said isoform having the following
5 characteristics: a) it is localized to neurons and nerve terminals; and
6 b) it is associated with synaptic vesicles; and
7 B. directions for use of said kit.

1 29. A test kit for demonstrating the presence of an isoform of synapsin III in
2 a eukaryotic cellular sample, comprising:
3 A. a predetermined amount of an isoform of synapsin III, said
4 isoform having the following characteristics:
5 a) it is localized to neurons and nerve terminals; and
6 b) it is associated with synaptic vesicles;
7 B. a predetermined amount of a specific binding partner of
8 said isoform;
9 C. other reagents; and
10 D. directions for use of said kit;
11 wherein either said isoform or said specific binding partner are detectably
12 labeled.

1 30. The test kit of Claim 28 or 29 wherein said labeled immunochemically
2 reactive component is selected from the group consisting of polyclonal antibodies
3 to the isoform, monoclonal antibodies to the isoform, fragments thereof, and
4 mixtures thereof.

1 31. A method of preventing and/or treating neurotransmitter debilitations,
2 derangements and/or dysfunctions and/or other disease states in mammals,

3 comprising administering to a mammal a therapeutically effective amount of a
4 material selected from the group consisting of an isoform of synapsin III, an
5 agent capable of promoting the production and/or activity of said isoform, an
6 agent capable of mimicking the activity of said isoform, an agent capable of
7 inhibiting the production of said isoform, and mixtures thereof, or a specific
8 binding partner thereto, said isoform having the following characteristics:
9 a) it is localized to neurons and nerve terminals; and
10 b) it is associated with synaptic vesicles.

1 32. The method of Claim 31 wherein said disease states include
2 schizophrenia.

1 33. The method of Claim 31 wherein said isoform is administered to
2 modulate the course of therapy where an isoform of synapsin III is being
3 administered as the primary therapeutic agent.

1 34. The method of Claim 31 wherein said isoform is administered to
2 modulate the course of therapy where said isoform is being co-administered with
3 one or more additional therapeutic agents.

1 35. A pharmaceutical composition for the treatment of cellular debilitation,
2 derangement and/or dysfunction in mammals, comprising:
3 A. a therapeutically effective amount of a material selected from
4 the group consisting of an isoform of synapsin III, an agent capable of promoting
5 the production and/or activity of said isoform, an agent capable of mimicking the
6 activity of said isoform, an agent capable of inhibiting the production of said
7 isoform, and mixtures thereof, or a specific binding partner thereto, said isoform
8 having the following characteristics:
9 a) it is localized to neurons and nerve terminals; and
10 b) it is associated with synaptic vesicles.

11 36. A purified isoform of synapsin III implicated in neurotransmission in
12 target cells in response to the isoform on said target cell, said isoform having the
13 following properties:

- 14 a) it is localized to neurons and nerve terminals; and
15 b) it is associated with synaptic vesicles.

1 37. A recombinant virus transformed with the DNA molecule, or a derivative
2 or fragment thereof, in accordance with Claim 14.

1 38. A recombinant virus transformed with the DNA molecule, or a derivative
2 or fragment thereof, in accordance with Claim 15.

1 39. A method of determining the synapsin III-related pharmacological activity
2 of a compound comprising:

- 3 administering the compound to a mammal;
4 determining the level of isoforms of synapsin III proteins present; and
5 comparing the level of isoforms of synapsin III to a standard.

1 40. The recombinant DNA molecule of Claim 16 comprising plasmid pET15b,
2 pGEX-3X, clone E3 or plasmid pGEX-3X, clone E4.

1 41. An isolated nucleic acid molecule encoding an isoform of synapsin III,
2 conserved variants thereof, or fragments thereof, wherein said isolated nucleic acid
3 molecule comprises a DNA sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID
4 NO:7, degenerate variants thereof, or fragments thereof.

1 42. The isolated nucleic acid molecule of Claim 41, wherein said isoform of
2 synapsin III is synapsin IIIa comprising an amino acid sequence of SEQ ID NO:1,
3 and said isolated nucleic acid molecule comprises a DNA sequence of SEQ ID
4 NO:2.

1 43. The isolated nucleic acid molecule of Claim 41, wherein said isoform of
2 synapsin III is synapsin IIIb comprising an amino acid sequence of SEQ ID NO:6,
3 and said isolated nucleic acid molecule comprises a DNA sequence of SEQ ID
4 NO:5.

1 44. The isolated nucleic acid molecule of Claim 41, wherein said isoform of
2 synapsin III is synapsin IIIc comprising an amino acid sequence of SEQ ID NO:8,
3 and said isolated nucleic acid molecule comprises a DNA sequence of SEQ ID
4 NO:7.

1 45. An isoform of a synapsin III protein wherein said isoform comprises an
2 amino acid sequence of SEQ ID NO:1, SEQ ID NO:6, or SEQ ID NO:8.

1 46. The isoform of claim 45, which is synapsin IIIa, and comprises an amino
2 acid sequence of SEQ ID NO:1.

1 47. The isoform of Claim 45 which is synapsin IIIb and comprises an amino
2 acid sequence of SEQ ID NO:6.

1 48. The isoform of Claim 45, which is synapsin IIIc and comprises an amino
2 acid sequence of SEQ ID NO:8.

Figure 1

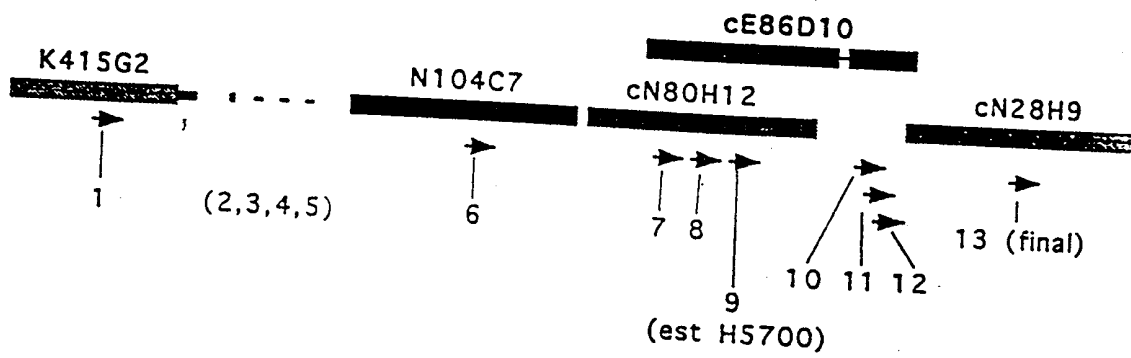


Figure 2

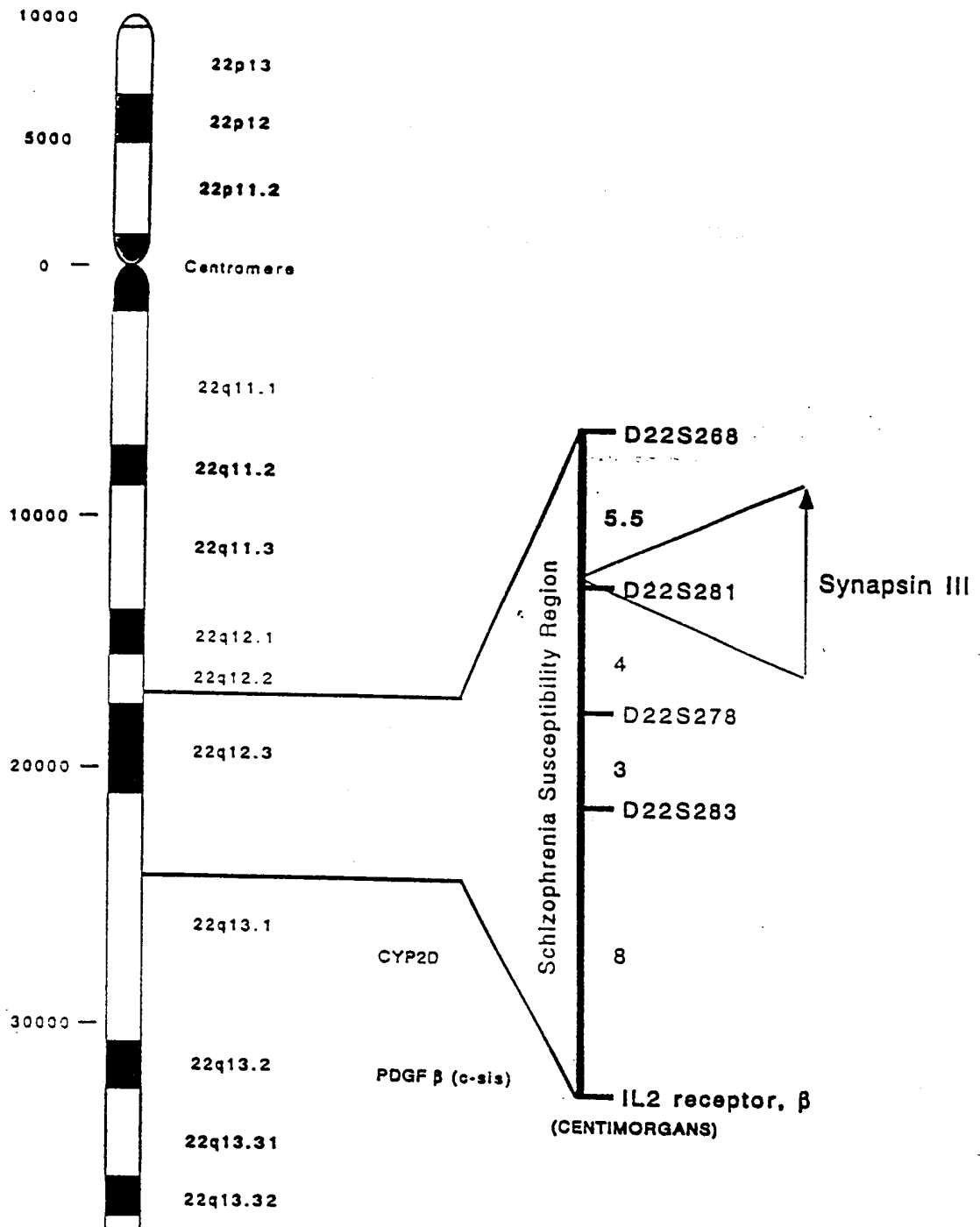


Figure 3

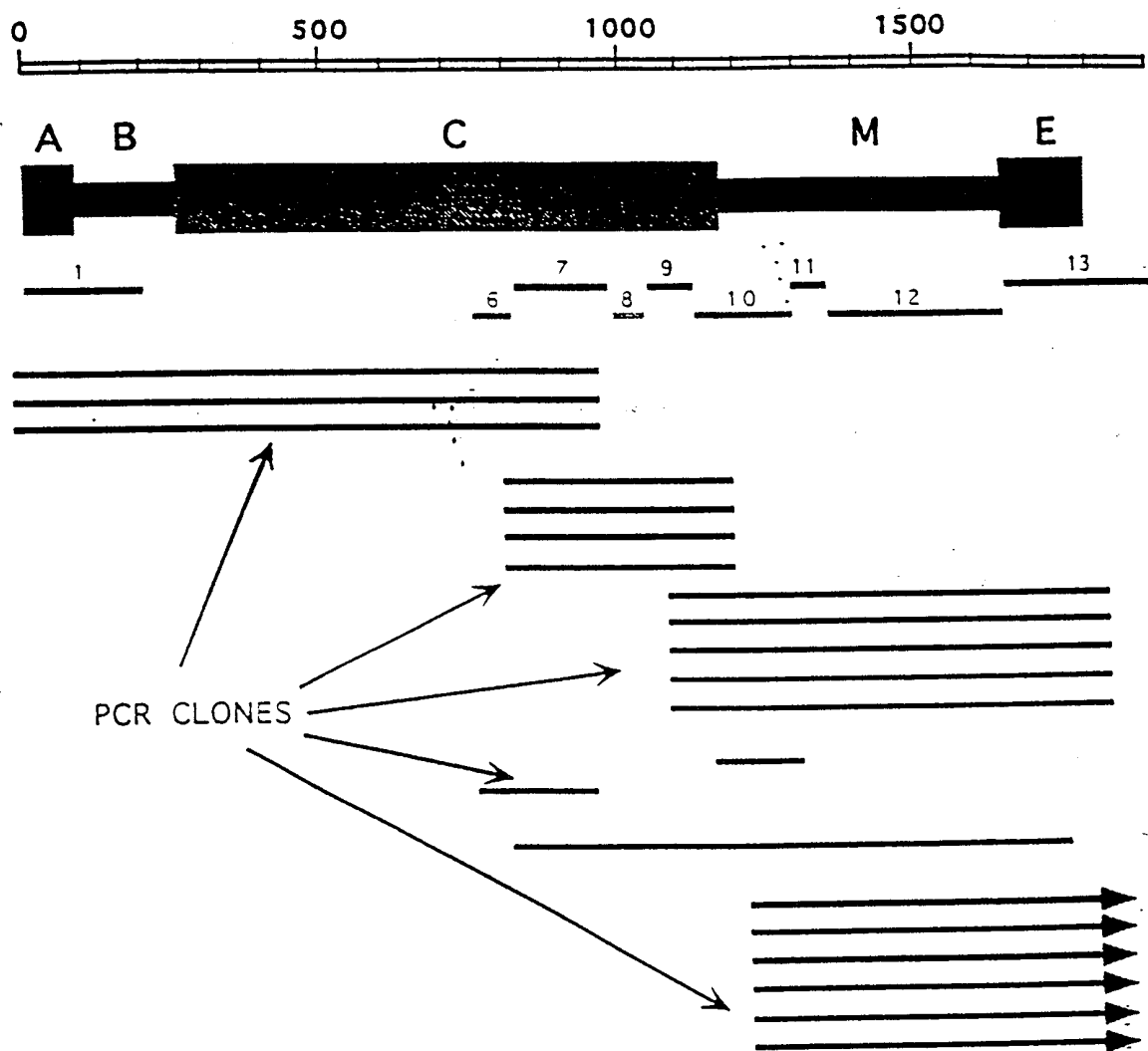


Figure 4

TGG GTA GGA GCC AGT CAT CTC CAT CCA TCC ACA GCC ATG AAT TTC CTC CGG CGA CGT CTC 60
M M F L R R R L

TCT GAC AGC AGC TTC ATG GCC AAC CTG CCT AAT GGC TAT ATG ACG GAC CTG CAA CGC CCA 120
S D S S F M A N L P M G Y M T D L Q R P

GAT AGC TCC ACC AGC TCA CCT GCT TCC CCC GCC ATG GAG AGG AGG CAC CCC CAG CCC CTG 180
D S S T S S P A S P A M E R R H P Q P L

GCT GCC TCC TTC TCC TCT CCA GGA TCC AGC CTT TTT AGC TCC CTC TCC AGT GCC ATG AAG 240
A A S F S S P G S S L F S S L S S A M K

CAG GCC CCT CAG GCC ACC TCA GGA CTG ATG GAG CCT CCA GGT CCC TCC ACG CCC ATT GTT 300
Q A P Q A T S G L M E P P G P S T P I V

CAA AGA CCC AGG ATC CTG TTG GTG ATC GAT GAT GCC CAT ACA GAC TGG TCG AAG TAT TTC 360
Q R P R I L L V I D D A H T D N S K Y F

CAT GGG AAG AAG GTG AAT GGA GAG ATT GAG ATC CGA GTG GAG CAG GCT GAA TTC TCA GAG 420
H G K K V N G E I E I R V E Q A E F S E

TTG AAC CTA GCT GCC TAT GTG ACC GGG GGC TGC ATG GTG GAC ATG CAG GTC GTG AGA AAT 480
L N L A A Y V T G G C H V D M Q V V R N

GGG ACC AAA GTG GTG AGC AGA TCC TTC AAG CCA GAC TTC ATC CTG GTC CGC CAG CAT GCC 540
G T K V V S R S F K P D F I L V R Q H A

TAC AGC ATG GCC CTG GGG GAA GAC TAC CGC AGC CTG GTC ATC GGC CTG CAG TAT GGA GGG 600
Y S M A L G E D Y R S L V I G L Q Y G G

CTG CCT GCT GTC AAC TCT CTC TAC TCC GTC TAC AAC TTC TGC AGC AAG CCC TGG GTG TTC 660
L P A V N S L Y S V Y N F C S K P W V F

TCT CAG CTC ATT AAG ATC TTC CAT TCC CTG GGT CCT GAG AAG TTC CCG CTT GTG GAG CAA 720
S Q L I K I F H S L G P E K F P L V E Q

ACA TTT TTC CCC AAC CAT AAG CCA ATG GTC ACA GCC CCA CAC TTC CCG GTG GTA GTC AAG 780
T F F P N H K P M V T A P H F P V V V K

CTG GGA CAT GCC CAC GCT GGA ATG GGA AAG ATC AAA GTG GAA AAC CAG CTT GAC TTC CAG 840
L G H A H A G M G K I K V E N Q L D F Q

GAC ATC ACC AGC GTG GTC GCC ATG GCC AAA ACA TAC GCC ACC ACC GAG GCG TTC ATC GAC 900
D I T S Y V A M A K T Y A T T E A F I D

TCC AAG TAC GAC ATC CGC ATC CAG AAA ATT GGA TCC AAC TAC AAG GCT TAC ATG AGA ACC 960
S K Y D I R I Q K I G S N Y K A Y M R T

TCC ATC TCT GGG AAC TGG AAG GCC AAC ACA GGC TCT GCC ATG CTG GAG CAG GTG GCC ATG 1020
S I S G N W K A N T G S A M L E Q V A M

ACA GAG AGG TAC AGG CTG TGG GTG GAC AGC TGC TCG GAA ATG TTT GGC GGC CTG GAC ATC 1080
T E R Y R L W V D S C S E M F G G L D I

TGT GCC GTC AAG GCT GTC CAC AGC AAG GAT GGC AGA GAT TAC ATC ATC GAG GTA ATG GAC 1140
C A Y K A V H S K D G R D Y I I E V M D

Figure 4 (Continued)

AGC TCA ATG CCG	CTG ATT GGA GAG CAT GTG GAA GAG GAC AGA CAG CTG ATG GCC GAC CTT	1200
S S M P	L I G E H V E E D R Q L M A D L	
GTT GTC TCC AAA	ATG AGC CAG CTC CCG ATG CCA GGA GGC ACA GCG CCC TCC CCE CTC AGA	1260
V V S K	M S Q L P H P G G T A P S P L R	
CCT TGG GCT CCA	CAG ATT AAA TCA GCG AAA TCC CCA GGG CAA GCC CAG CTG GGG CCT CAG	1320
P W A P	Q I K S A K S P G Q A Q L G P Q	
CTA RGC CAG CCC	CAG CCA GCG CCA CCT CCG CAA GGA GGC CCT CGC CAA GCT CAG TCT CCT	1380
L X Q P	Q P R P P P Q G G P R Q A Q S P	
CAG CCC CAG AGA TCT GGA AGC CCC TCC CAA CAG AGG CTC TCC CCA CAA GGC CAG CAG CCC		1440
Q P Q R S	G S S P S Q Q R L S P Q G Q Q P	
CTG AGC CCC CAG TCC GGA TCT CCA CAG CAG CAA AGG TCA CCA GGC TCT CCG CAG CTA TCC		1500
L S P Q S	G S S P Q Q Q R S P G S P Q L S	
CGG GCA TCC AGT	GGC AGC TCC CCA AAC CAR GCC TCC AAG CCA GGT GCC ACC CTC GCC TCA	1560
R A S S	G S S P H Q A S K P G A T L A S	
CAG CCC CGG CCC	CCT GTG CAG GGC CGT AGT ACC TCC CAG CAG GGT GAA GAG TCC AAG AAG	1620
Q P R P P	P V Q G R S T S Q Q G E E S K K	
CCA GCA CCA CCC	CAT CCG CAT CTC AAC AAA TCT CAG TCC CTG ACT AAC AGC CTC AGC ACA	1680
P A P P H	P H L N K S Q S L T N S L S T	
TCC GAC ACC TCC	CAG CGT GGG ACC CCA AGT GAA GAC GAG GCC AAG GCT GAA ACC ATC CGC	1740
S D T S Q	R G T P S E D E A K A E T I R	
AAC CTG AGG AAG	TCT TTT GCC AGC CTG TTC TCT GAC TAA CGC CAT CCA GGC TGG GAG GGG	1800
N L R K S	S F A S L F S D	
AAG AGT GCT ATG	GTA CAC TCG TCC CCY TCC TGC CTC ATC TTC CTT CTC AGC CTT GGT TCC	1860
TGA TGG GAA CAG	AAT GGA GGG CCT GAG AAC ATA CTT TCT AAA TGC CTT TGA CCC AGG AAC	1920
CGA TTA TCT ATA	TTT GTT CCC ATT TTC CTT CAC CGT GAC ATT CCA GCA TTG TCT GAC TGT	1980
GAG GTG GGC CTT	TGA GAG CCT CCA GGT TCC TCA AAA CAG GCC TGA GCG ATG GGC ATC ACA	2040
CCC TCT GCC TAC	CCA CGT GCA TGC TTA CCT GCC AGA TAA CCA AGT GAG ATG TCT GCG AGT	2100
GGC TAG TTT TCA	CAT TCT TAC TAG TGT TTG GCT CAC CTT TGG GCA AAG GCC CCY TTA GGC	2160
CTT GCC CCA CCT	CCA TCA AAC GCA GAC ACT GTA GTC AGA CCT CAG CAA TAT AGG AGG CAA	2220
TAA TCT TTT AAC	AGT GTT TTG CAA ACA AAC AAA AAA AAA AA	

Figure 5

IIIa	PPFLARRLS	QSEFMANLFN	GYTDLQRG	-----	-----SST	11
IIa	PPFLARRLS	QSEFMANLFN	GYTDLQRG	PQQPPPPPPP	GPAAASAA	50
Ia	PPFLARRLS	QSEFMANLFN	GYTDLQRG	PPPPPP----	--GAHSPGAT	41
IIIa	SSPASPAMR	RH--PQPLAA	SFSST---	ESLFSLSLDA	IKQLPCATS-	75
IIa	PPASFGRR	RP-PPASAA	ACPAPPSLG	ESLFSLSLDA	IKQLTAASAG-	98
Ia	PPGQACRR	SSGVAASG	AAPSSSGG	ESLFSLSLDA	IKQLTAAAA	91
IIIa	GLNEPPGPT	PTVQ----	RLLVLEERH	IKWAKMENGK	KLHCEITIM	121
IIa	LVDAAPAP	-AAAH---	RLLVLEERH	IKWAKMENGK	KLHCEITIM	143
Ia	TFSEVGGGS	GGACGGAA	RLLVLEERH	IKWAKMENGK	KLHCEITIM	143
IIIa	EAETSENL	WVAVLQV	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	171
IIa	EAETSENL	WVAVLQV	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	192
Ia	EAETSENL	WVAVLQV	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	192
IIIa	ALGSPSEV	ELQVHCLF	INSTLSMIF	QIKWVTHCL	VLRKQCLDE	221
IIa	ALGSPSEV	ELQVHCLF	INSTLSMIF	QIKWVTHCL	VLRKQCLDE	242
Ia	ALGSPSEV	ELQVHCLF	INSTLSMIF	QIKWVTHCL	VLRKQCLDE	242
IIIa	EPFLQCTFY	PNQKMLTLP	TRVWVNGH	AHEGKQIKV	ENLDFQDIA	271
IIa	EPFLQCTFY	PNQKMLTLP	TRVWVNGH	AHEGKQIKV	ENLDFQDIA	292
Ia	EPFLQCTFY	PNQKMLTLP	TRVWVNGH	AHEGKQIKV	ENLDFQDIA	292
IIIa	SVYALQYA	TRSEIDKY	QVLRKQIK	YKAVRTSIS	GNWQNTGSA	321
IIa	SVYALQYA	TRSEIDKY	QVLRKQIK	YKAVRTSIS	GNWQNTGSA	342
Ia	SVYALQYA	TRSEIDKY	QVLRKQIK	YKAVRTSIS	GNWQNTGSA	342
IIIa	MESQANER	WVAVLQV	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	371
IIa	MESQANER	WVAVLQV	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	392
Ia	MESQANER	WVAVLQV	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	392
IIIa	PLDEAIED	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	ANLKSASG	420
IIa	PLDEAIED	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	ANLKSASG	440
Ia	PLDEAIED	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	ANLKSASG	442
IIIa	Q---LALQP	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	QVLRKQIK	457
IIa	Q---LALQP	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	QVLRKQIK	470
Ia	Q---LALQP	QVLRKQIK	VLRSEAPCF	ILLRCHAFS	QVLRKQIK	491
IIIa	PL-----	-S-PC-	-----	-----	-----SG	474
IIa	PL-----	-S-PC-	-----	-----	-----SG	482
Ia	PL-----	-S-PC-	-----	-----	-----SG	541
IIIa	EPQCPSPGS	P-----	-----	-----	-----QLS	488
IIa	EPQCPSPGS	P-----	-----	-----	-----QLS	498
Ia	EPQCPSPGS	P-----	-----	-----	-----QLS	591
IIIa	PASSCS	-----	-----	-----	-----SG	519
IIa	PASSCS	-----	-----	-----	-----SG	525
Ia	PASSCS	-----	-----	-----	-----SG	641
IIIa	SCQGESKCP	A--RPHFN	KSQSLNLS	TSQTS-CPIT	PSDEPKAET	566
IIa	SCQGESKCP	A--RPHFN	KSQSLNLS	TSQTS-CPIT	PSDEPKAET	568
Ia	SCQGESKCP	A--RPHFN	KSQSLNLS	TSQTS-CPIT	PSDEPKAET	682
IIIa	IRLRKSFAS	LFSD	-----	-----	-----	580
IIa	IRLRKSFAS	LFSD	-----	-----	-----	582
Ia	IRLRKSFAS	LFSD	-----	-----	-----	696

Figure 6a

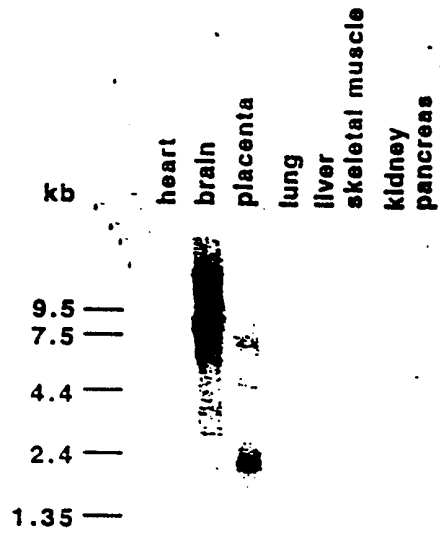


Figure 6b

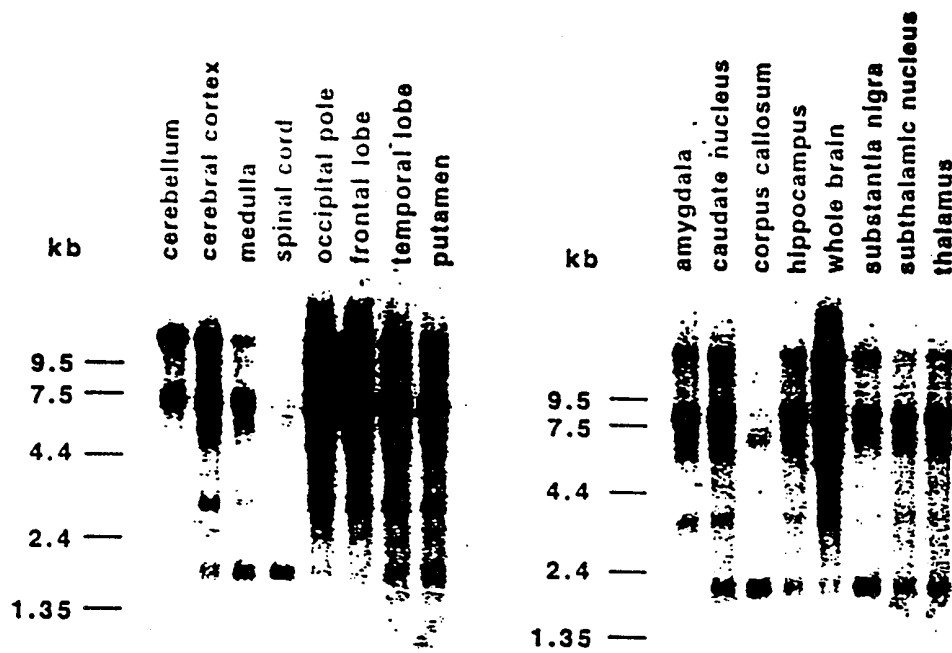


Figure 7A

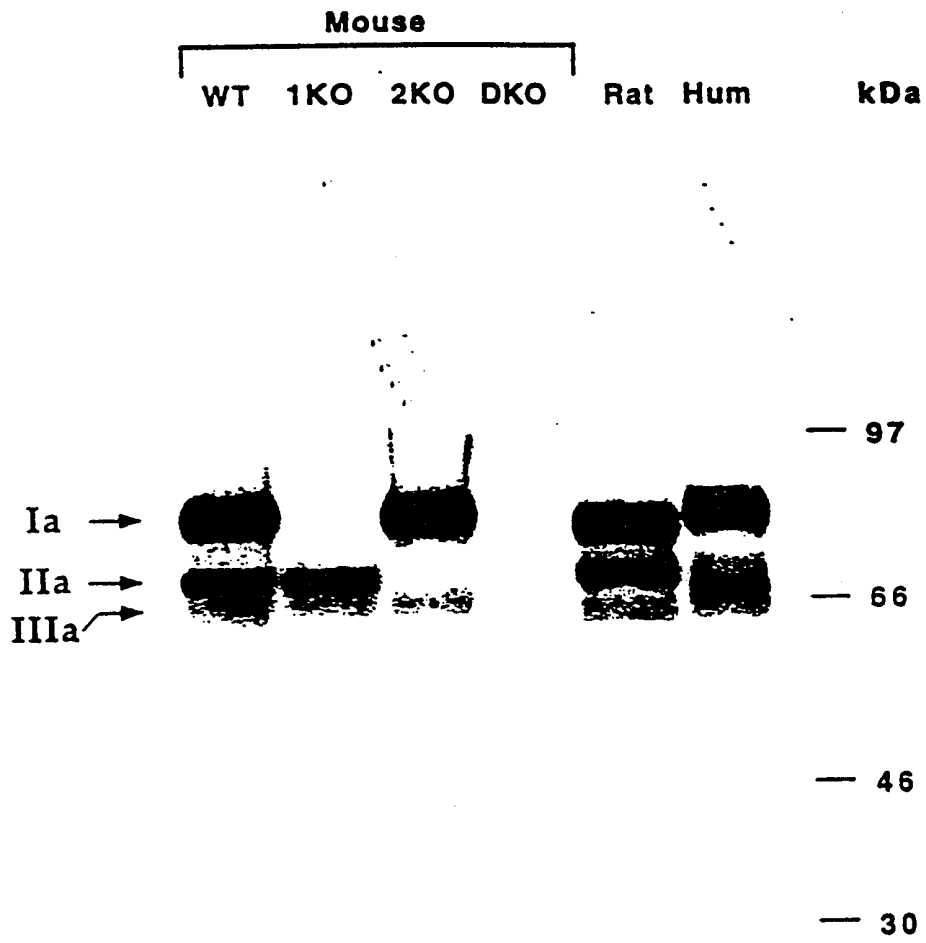


Figure 7B

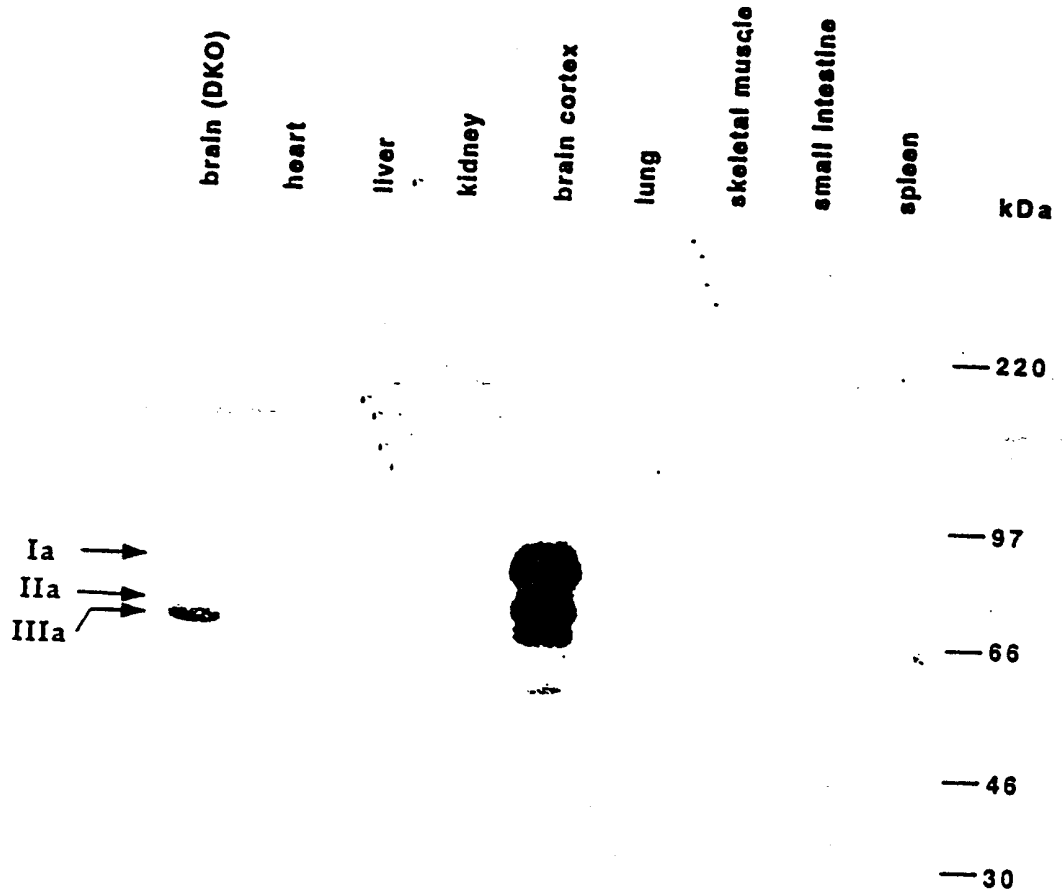


Figure 8

MNFLRRRLSDSSFMANLFGYMTDLQRDSTSSPASPAMERRHPQPLAASFSSPGSSLFSSLSAMKQAPQATSGLMEP
PGPSTPIVQRPRILLVIDDAHTDWSKYFHGKKNVGEIEIRVEQAEFSELNLAAYVTGGCMVDMQVVRNGTKVVSRSFKPD
FILVRQHAYSMALGEDYRSLVIGLQYGGLPVNSLYSVNFCSKPWFSQLIKIFHSLGPEKFFLVBQTFEENHKFMVTA
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30

Ser Ser Pro Ala Ser Pro Ala Met Glu Arg Arg His Pro Gln Pro Leu

35

40

45

Ala Ala Ser Phe Ser Ser Pro Gly Ser Ser Leu Phe Ser Ser Leu Ser

50

55

60

Ser Ala Met Lys Gln Ala Pro Gln Ala Thr Ser Gly Leu Met Glu Pro

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70

75

80

Pro Gly Pro Ser Thr Pro Ile Val Gln Arg Pro Arg Ile Leu Leu Val

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90

95

Ile Asp Asp Ala His Thr Asp Trp Ser Lys Tyr Phe His Gly Lys Lys

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105

110

Val Asn Gly Glu Ile Glu Ile Arg Val Glu Gln Ala Glu Phe Ser Glu

115

120

125

Leu Asn Leu Ala Ala Tyr Val Thr Gly Gly Cys Met Val Asp Met Gln

130

135

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Val Val Arg Asn Gly Thr Lys Val Val Ser Arg Ser Phe Lys Pro Asp

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150

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160

Phe Ile Leu Val Arg Gln His Ala Tyr Ser Met Ala Leu Gly Glu Asp

165

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Tyr Arg Ser Leu Val Ile Gly Leu Gln Tyr Gly Gly Leu Pro Ala Val

180

185

190

Asn Ser Leu Tyr Ser Val Tyr Asn Phe Cys Ser Lys Pro Trp Val Phe

195

200

205

Ser Gln Leu Ile Lys Ile Phe His Ser Leu Gly Pro Glu Lys Phe Pro

210

215

220

Leu Val Glu Gln Thr Phe Phe Pro Asn His Lys Pro Met Val Thr Ala

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240

Pro His Phe Pro Val Val Val Lys Leu Gly His Ala His Ala Gly Met

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255

Gly Lys Ile Lys Val Glu Asn Gln Leu Asp Phe Gln Asp Ile Thr Ser

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265

270

Val Val Ala Met Ala Lys Thr Tyr Ala Thr Thr Glu Ala Phe Ile Asp

275

280

285

Ser Lys Tyr Asp Ile Arg Ile Gln Lys Ile Gly Ser Asn Tyr Lys Ala

290

295

300

Tyr Met Arg Thr Ser Ile Ser Gly Asn Trp Lys Ala Asn Thr Gly Ser

305

310

315

320

Ala Met Leu Glu Gln Val Ala Met Thr Glu Arg Tyr Arg Leu Trp Val

325

330

335

Asp Ser Cys Ser Glu Met Phe Gly Gly Leu Asp Ile Cys Ala Val Lys

340

345

350

Ala Val His Ser Lys Asp Gly Arg Asp Tyr Ile Ile Glu Val Met Asp

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360

365

Ser Ser Met Pro Leu Ile Gly Glu His Val Glu Glu Asp Arg Gln Leu

370

375

380

Met Ala Asp Leu Val Val Ser Lys Met Ser Gln Leu Pro Met Pro Gly

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390

395

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Gly Thr Ala Pro Ser Pro Leu Arg Pro Trp Glu Ala Leu Ala Lys Leu

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Pro His Lys Ala Ser Ser Pro

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<212> PRT

<213> Homo sapiens

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Leu Pro Asn Gly Tyr Met Thr Asp Leu Gln Arg Pro Asp Ser Ser Thr

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25

30

Ser Ser Pro Ala Ser Pro Ala Met Glu Arg Arg His Pro Gln Pro Leu

35

40

45

Ala Ala Ser Phe Ser Ser Pro Gly Ser Ser Leu Phe Ser Ser Leu Ser

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60

Ser Ala Met Lys Gln Ala Pro Gln Ala Thr Ser Gly Leu Met Glu Pro

65

70

75

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Pro Gly Pro Ser Thr Pro Ile Val Gln Arg Pro Arg Ile Leu Leu Val

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Ile Asp Asp Ala His Thr Asp Trp Ser Lys Tyr Phe His Gly Lys Lys

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Val Asn Gly Glu Ile Glu Ile Arg Val Glu Gln Ala Glu Phe Ser Glu

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Leu Asn Leu Ala Ala Tyr Val Thr Gly Gly Cys Met Val Asp Met Gln

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Val Val Arg Asn Gly Thr Lys Val Val Ser Arg Ser Phe Lys Pro Asp

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155

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Phe Ile Leu Val Arg Gln His Ala Tyr Ser Met Ala Leu Gly Glu Asp

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175

Tyr Arg Ser Leu Val Ile Gly Leu Gln Tyr Gly Gly Leu Pro Ala Val

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190

Asn Ser Leu Tyr Ser Val Tyr Asn Phe Cys Ser Lys Pro Trp Val Phe

195

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205

Ser Gln Leu Ile Lys Ile Phe His Ser Leu Gly Pro Glu Lys Phe Pro

210

215

220

Leu Val Glu Gln Thr Phe Phe Pro Asn His Lys Pro Met Val Thr Ala

225

230

235

240

Pro His Phe Pro Val Val Val Lys Leu Gly His Ala His Ala Gly Met

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Gly Lys Ile Lys Val Glu Asn Gln Leu Asp Phe Gln Asp Ile Thr Ser

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Val Val Ala Met Ala Lys Thr Tyr Ala Thr Thr Glu Ala Phe Ile Asp

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Ser Lys Tyr Asp Ile Arg Ile Gln Lys Ile Gly Ser Asn Tyr Lys Ala

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Tyr Met Arg Thr Ser Ile Ser Gly Asn Trp Lys Ala Asn Thr Gly Ser

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Ala Met Leu Glu Gln Val Ala Met Thr Glu Arg Tyr Arg Leu Trp Val

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Asp Ser Cys Ser Glu Met Phe Gly Gly Leu Asp Ile Cys Ala Val Lys

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345

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Ala Val His Ser Lys Asp Gly Arg Asp Tyr Ile Ile Glu Val Met Asp

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Ser Ser Met Pro Leu Ile Gly Glu His Val Glu Glu Asp Arg Gln Leu

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Met Ala Asp Leu Val Val Ser Lys Met Ser Gln Leu Pro Met Pro Gly

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395

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Gly Thr Ala Pro Ser Pro Leu Arg Pro Trp Ala Pro Gln Ile Lys Ser

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Ala Lys Ser Pro Gly Gln Ala Gln Leu Gly Pro Gln Leu Gly Gln Pro

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Gln Pro Arg Pro Pro Pro Gln Ala Asn Leu Ser Pro

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440