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(54) **METHODS OF IDENTIFYING AGENTS THAT AFFECT CLEAVAGE OF AMYLOID-BETA PRECURSOR PROTEIN**

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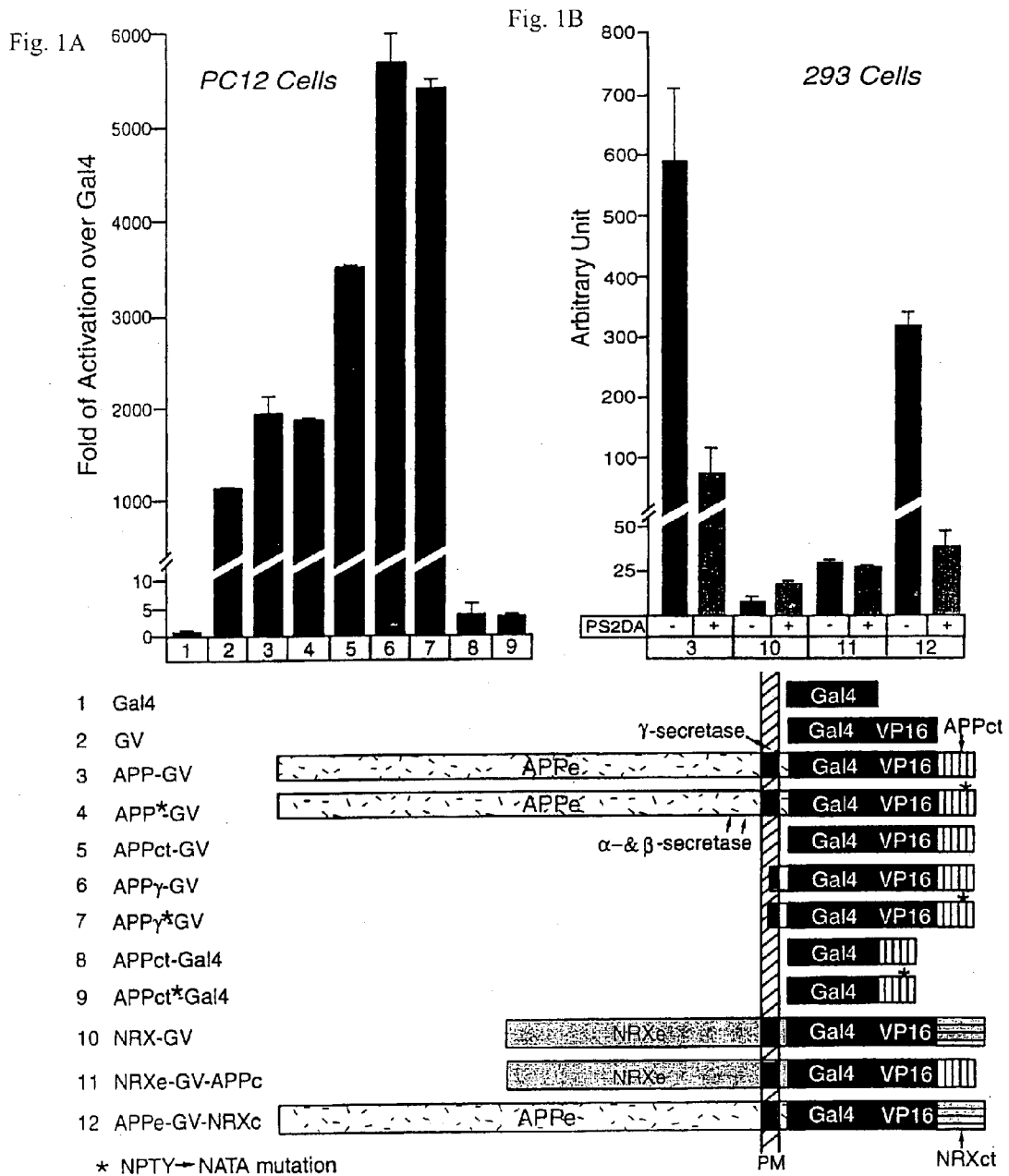
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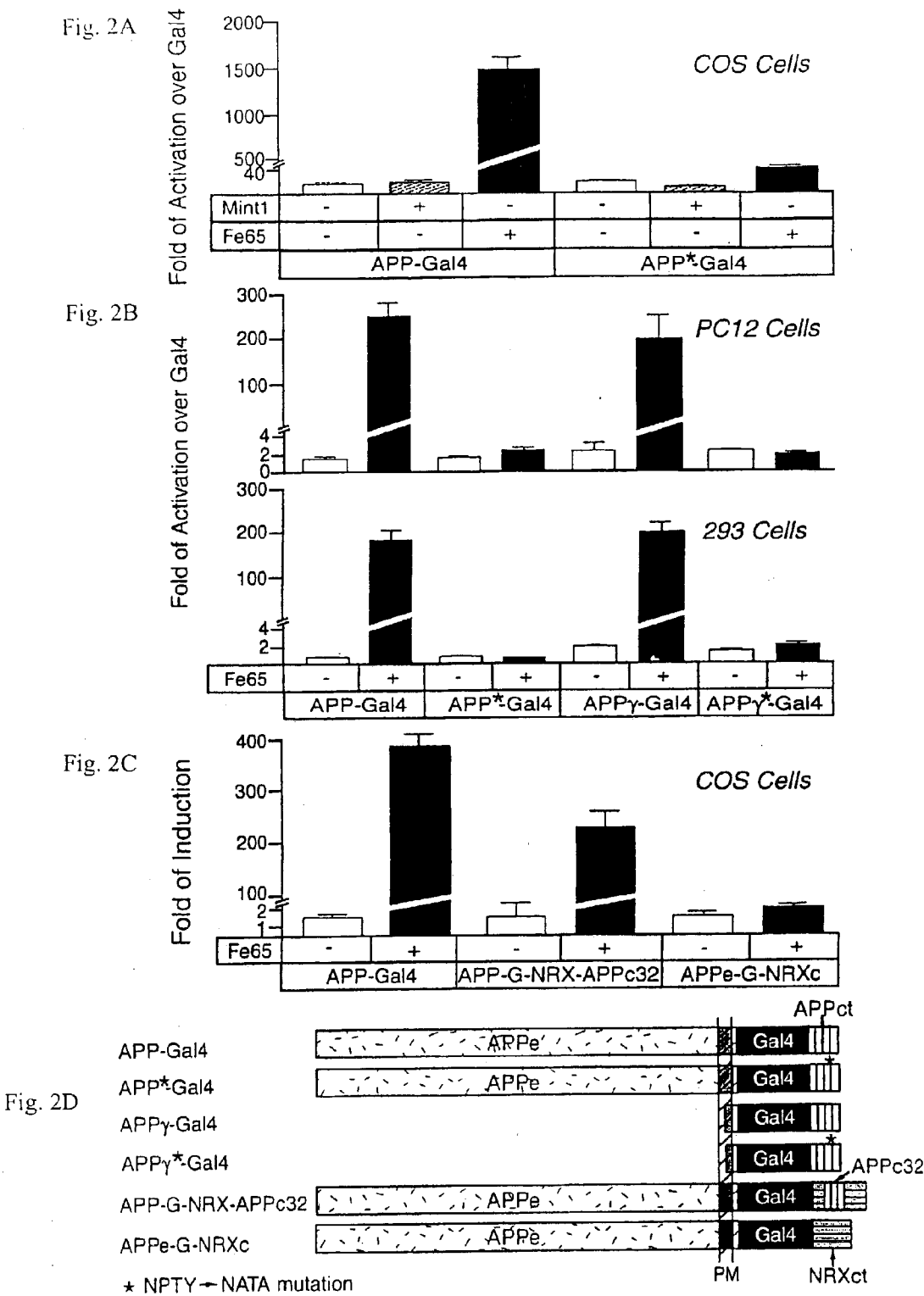
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ABSTRACT

The present invention provides methods of identifying agents that affect the cleavage of amyloid- β precursor protein (APP) and related vectors, cells and kits, as well as agents identified by the method.





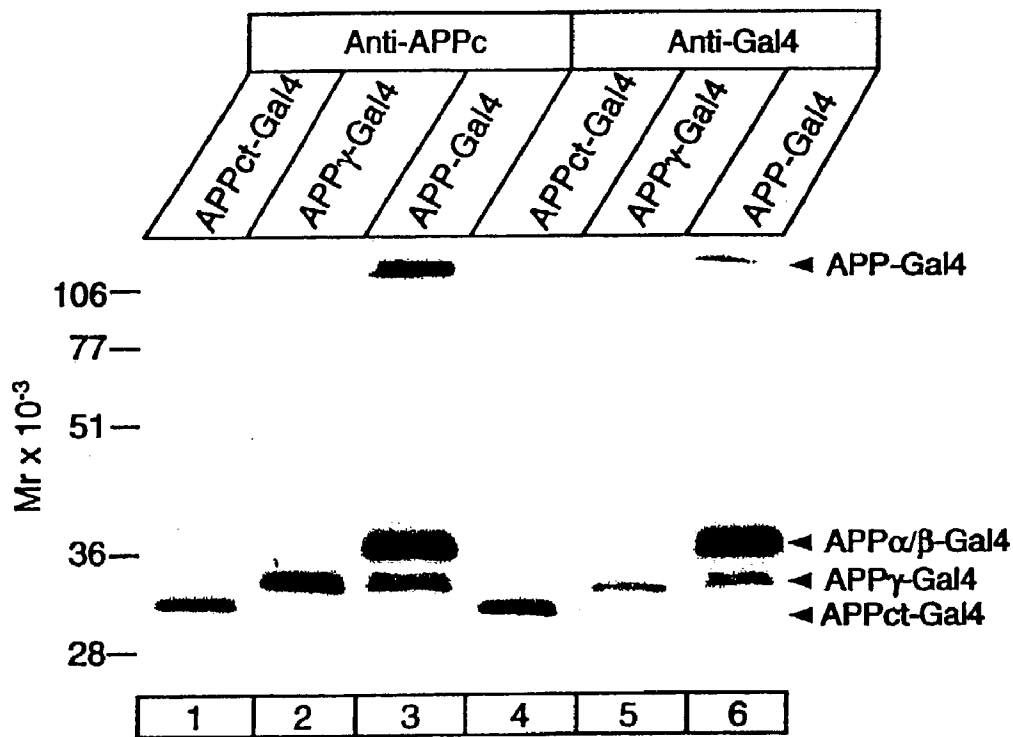
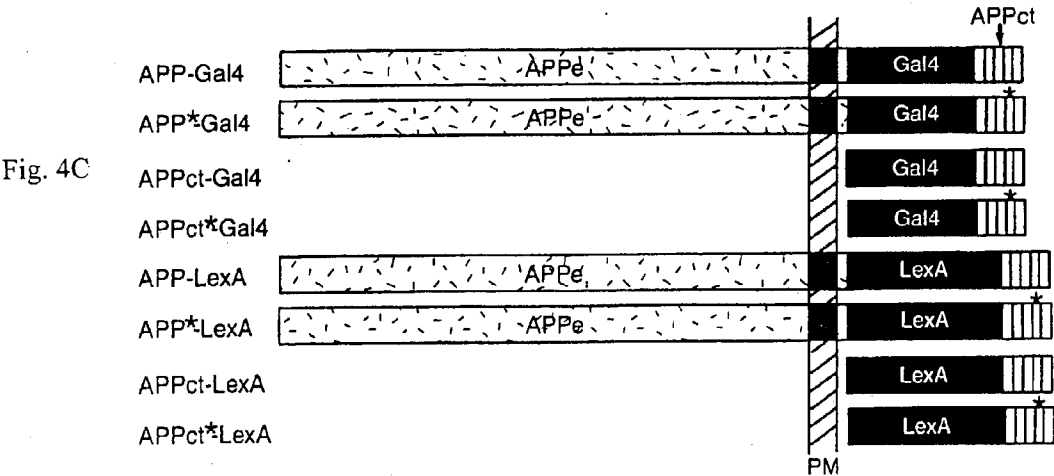
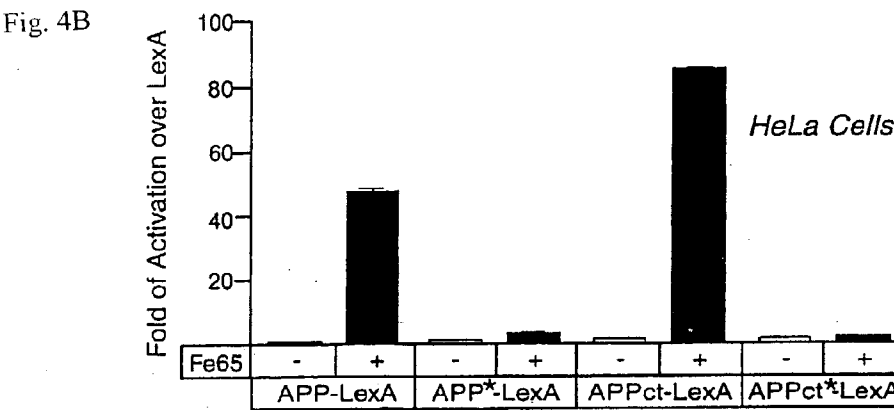
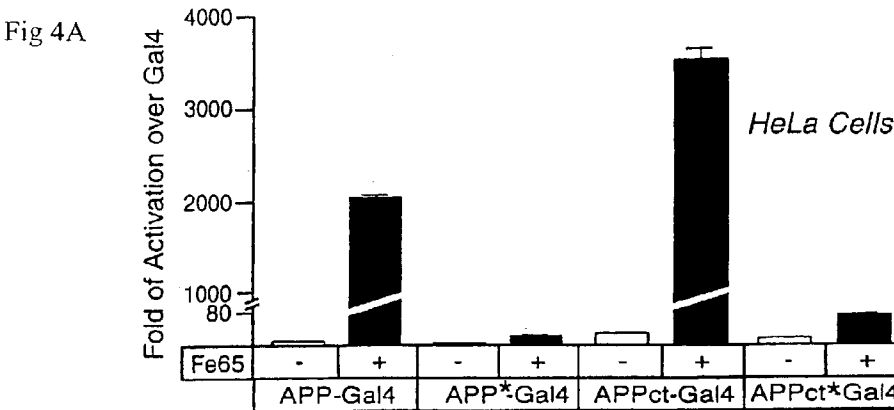
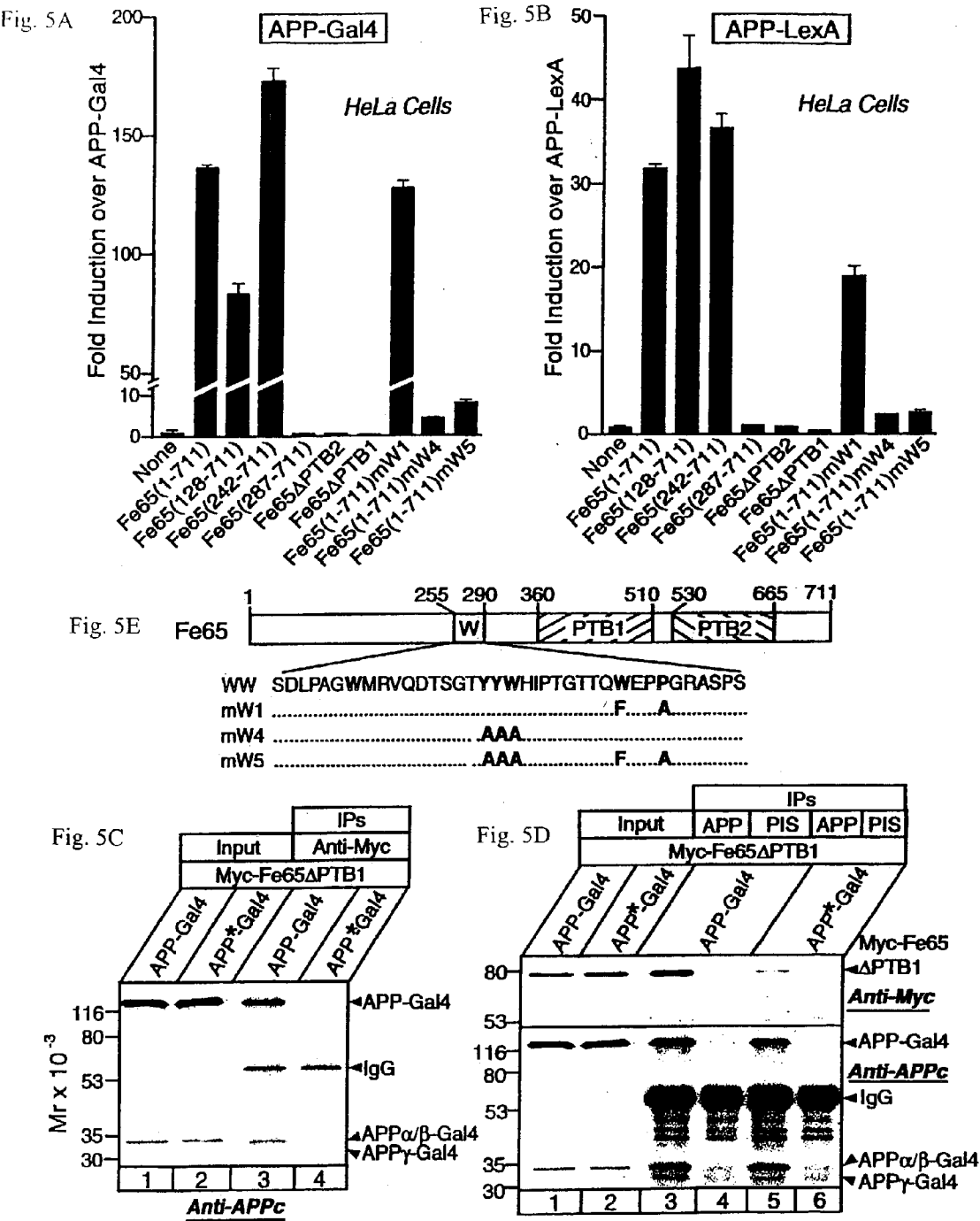


Fig. 3





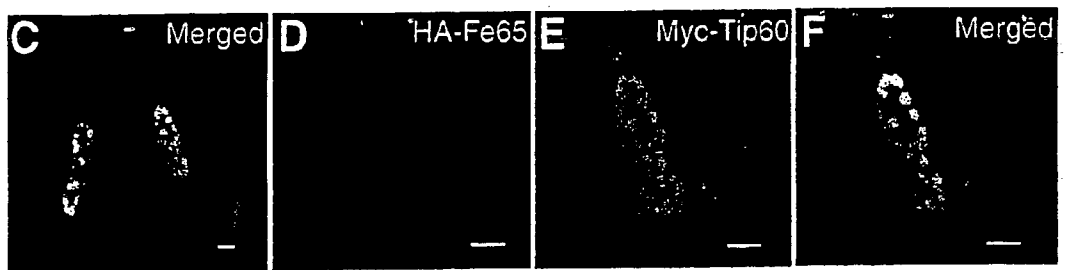
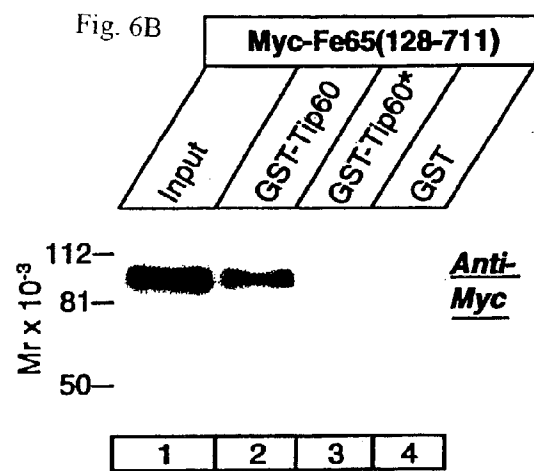
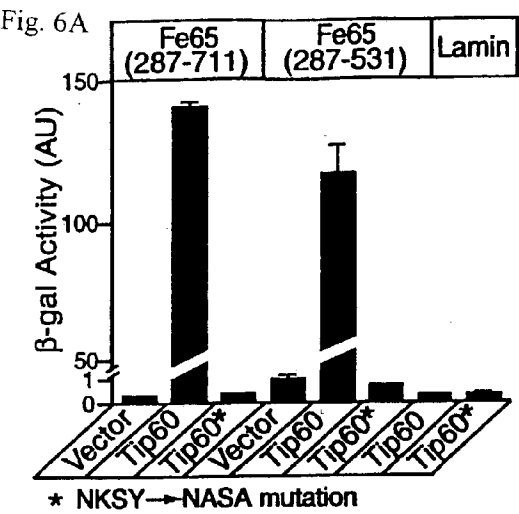
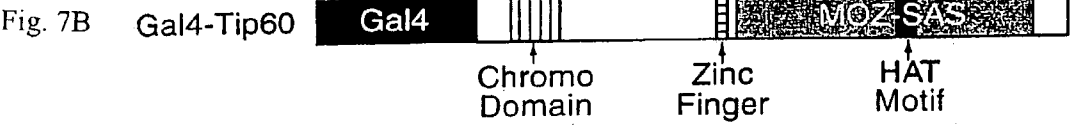
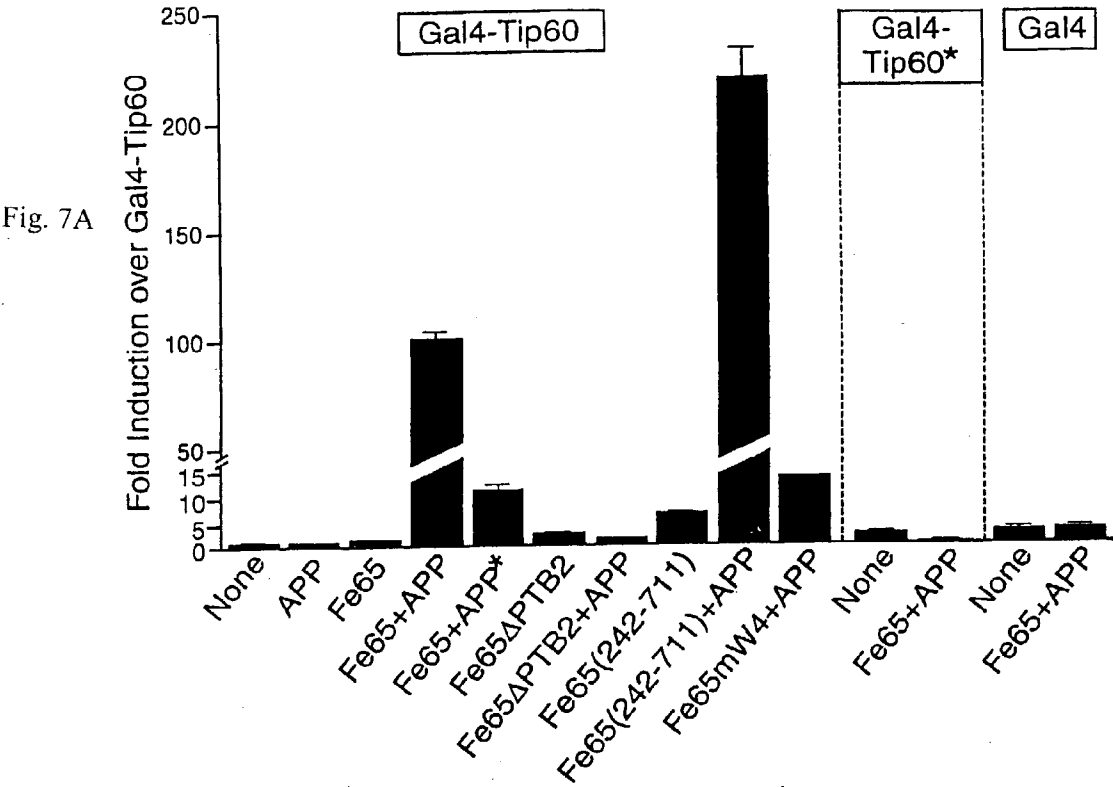


Fig. 6C

Fig. 6D

Fig. 6E

Fig. 6F



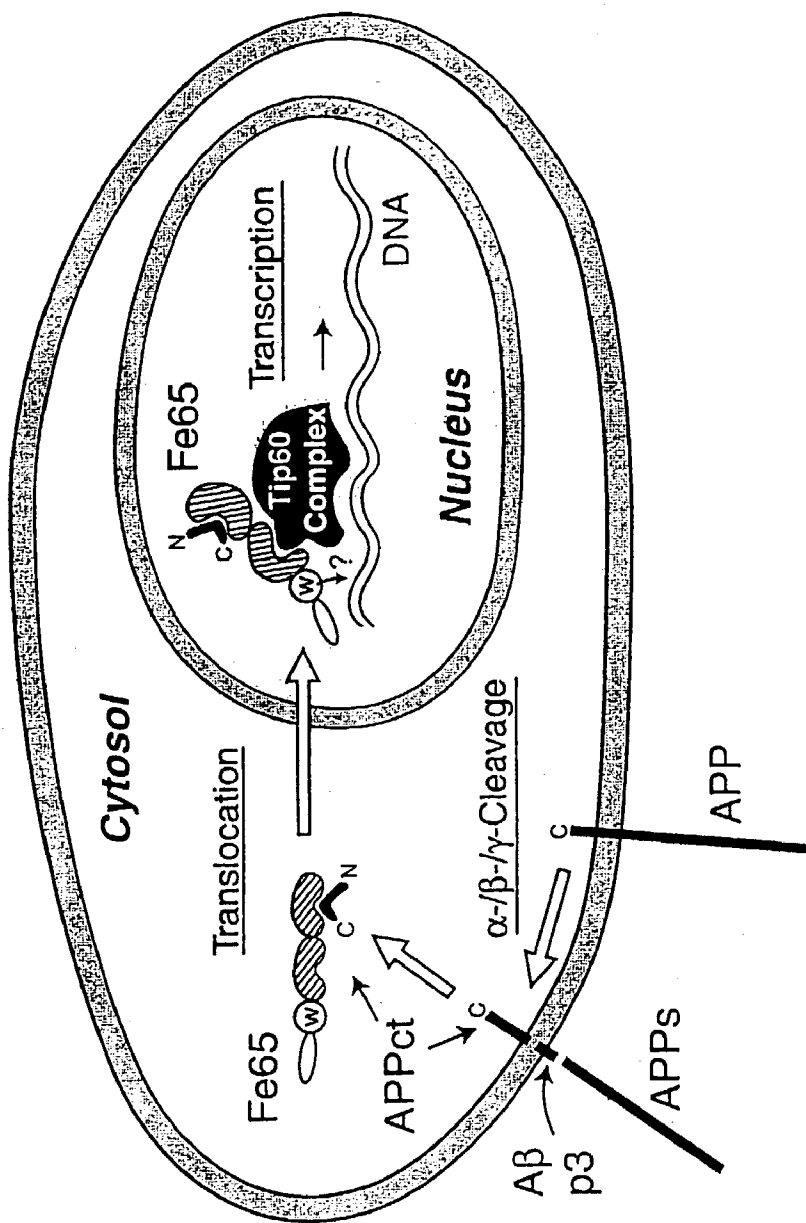


Fig. 8

METHODS OF IDENTIFYING AGENTS THAT AFFECT CLEAVAGE OF AMYLOID-BETA PRECURSOR PROTEIN

BACKGROUND OF THE INVENTION

[0001] Alzheimer's disease is a degenerative brain disorder that is characterized clinically by progressive loss of memory and cognitive impairment. Pathologically, the disease is characterized by lesions comprising neurofibrillary tangles, cerebrovascular amyloid deposits, and neuritic plaques. The cerebrovascular amyloid deposits and neuritic plaques contain amyloid- β peptide. The aggregation of amyloid- β peptide is instrumental in the pathogenesis of Alzheimer's disease.

[0002] Amyloid- β peptide is derived from amyloid- β precursor protein (APP). APP is a cell-surface protein with a large N-terminal extracellular sequence, a single transmembrane region (TMR) and a short C-terminal cytoplasmic tail. APP is processed by proteolysis in all cells. Initially, α - and β -secretases cleave APP at defined extracellular sequences just outside of the TMR to release a large N-terminal extracellular fragment. Thereafter, γ -secretase cuts APP in the middle of the TMR to generate small extracellular peptides and a C-terminal fragment comprising half of the TMR and the full cytoplasmic tail. See, e.g., Selkoe (1998) Trends Cell Biol. 8, 447-453; Bayer et al. (1999) Mol. Psychiatry 4, 524; Haass et al. (1999) Science 286, 916-919; Price et al. (1998) Ann. Rev. Genet 32, 461-493

[0003] Cleavage of APP by β - and γ -secretase produces the amyloid- β peptides (AP40 and AP42) implicated in the pathogenesis of Alzheimer's disease. Various methods for the diagnosis and monitoring of the disease involve assessing the cleavage of APP and detection of amyloid- β peptide. However, these methods suffer from various disadvantages including the insolubility of amyloid- β peptide, cross-reactivity of antibodies against the peptide with the precursor APP, and different levels of protease activity in different body fluids.

[0004] The γ -cleavage of APP is mediated by presenilins, intrinsic membrane proteins that may correspond to γ -secretase and that are mutated in some cases of familial Alzheimer's disease. See, e.g., Esler et al. (2000) Nat. Cell. Biol. 2, 428-434. Also, γ -cleavage occurs in APP-homologs that are not implicated in Alzheimer's disease. For example, Notch proteins are membrane proteins that are also cleaved in the middle of the TMR in a presenilin-dependent reaction. See, e.g. Ye et al. (1999) Nature 398, 525-529; De Strooper et al. (1999) Nature 398, 518-522; Struhl et al. (1999) Nature 398, 522-525. Notch proteins are cell-surface proteins involved in intercellular signaling in which presenilin-dependent cleavage liberates a cytoplasmic fragment that functions in nuclear transcription. Struhl et al. (2000) Mol. Cell 6, 625-636. Sterol regulatory element binding proteins (SREBPs) are also cleaved in the TMRs to generate nuclear transcription factors. Brown et al. (2000) Cell 100, 391-398. In contrast, the physiological significance of γ -cleavage of APP, and in particular the biological role of the cytoplasmic tail fragment, has heretofore been unclear. In accordance with the present invention it has been discovered that the cytoplasmic tail forms a functional complex with nuclear proteins, and that the complex is a potent stimulator of transcription. The present invention thus provides new methods for identifying agents that affect the cleavage of APP.

SUMMARY OF THE INVENTION

[0005] The present invention provides a method of identifying an agent that affects the cleavage of APP comprising contacting a cell containing APP modified in the C-terminal cytoplasmic tail to allow detection of nuclear localization with a candidate agent and measuring nuclear localization of a C-terminal cytoplasmic cleavage product of APP in the presence and absence of the agent.

[0006] In another embodiment, the present invention provides a method of identifying an agent that affects the cleavage of APP comprising providing a cell containing APP and a protein that interacts with the C-terminal cytoplasmic cleavage product of APP to regulate transcription, wherein the protein is modified to allow detection of nuclear localization of the C-terminal cytoplasmic cleavage product of APP; contacting the cell with a candidate agent; and measuring nuclear localization of the C-terminal cytoplasmic cleavage product of APP in the presence and absence of the agent.

[0007] The invention further provides agents identified by the foregoing method, and compositions comprising the agents.

[0008] In another embodiment, the invention is directed to vectors, transfected cells and kits useful for identifying an agent that affects the cleavage of APP.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIGS. 1A-C show results of Gal4-transactivation assays in PC12 cells (FIG. 1A) and HEK293 cells (FIG. 1B) and the domain structures of proteins encoded by the test plasmids (FIG. 1C). Test plasmids are identified by numbers below the bars in FIGS. 1A and 1B. Luciferase activity was normalized for β -galactosidase activity to control for translation efficiency. In FIG. 1A, luciferase activity was additionally normalized for the activity of cells co-transfected with Gal4 alone. FIGS. 1A and 1B show results from representative experiments.

[0010] FIGS. 2A-D show results of Gal4-transactivation assays (FIGS. 2A-C) obtained with the constructs depicted (FIG. 2D). All bar diagrams exhibit representative experiments in the cell types identified to the right of the panel.

[0011] FIG. 3 depicts an immunoblot of COS cells transfected with APP-Gal4 fusion proteins. The positions of full-length APP-Gal4, the α / β - and γ -cleavage products of APP-Gal4 (APP α / β -Gal4 and APP γ -Gal4, respectively) and the cytoplasmic tail of APP-Gal4 (APPct-Gal4) are indicated on the right. Numbers on the left show positions of molecular weight markers.

[0012] FIGS. 4A-C show transactivation of transcription measured with Gal4-fusion proteins and a Gal4-dependent reporter plasmid (FIG. 4A) as compared with transactivation obtained with LexA-fusion proteins and a LexA-dependent reporter plasmid (FIG. 4B). The structures of the proteins co-expressed with the reporter plasmids and the control β -galactosidase vector are shown in FIG. 4C. Transcriptional activation is expressed as fold increase over transcription obtained with the DNA-binding proteins expressed alone.

[0013] FIGS. 5A-E show transactivation using full length APP-Gal4 (FIG. 5A) and APP-LexA (FIG. 5B) fusion

proteins co-transfected with corresponding reporter plasmids and a β -galactosidase control plasmids. Various Fe65 proteins described below the bar diagrams were co-transfected with the test and control plasmids. Transactivation is expressed as fold induction over transfection with Gal4-APP or LexA-APP alone. The domain structure of Fe65 and deletion mutants of Fe65 are shown in FIG. 5E. FIGS. 5C and D show results of immunoprecipitations carried out with antibodies to the myc-epitope (FIG. 5C) or to APP (FIG. 5D; PIS=preimmune serum) and analyzed by immunoblotting with antibodies to APP (FIG. 5C and lower part of FIG. 5D) or to myc (upper part of FIG. 5D). The positions of the various proteins are indicated on the right of the immunoblots, and the locations of molecular weight standards are shown on the left.

[0014] FIGS. 6A-F demonstrate the interaction of Fe65 with Tip60. FIG. 6A shows results of a quantitative yeast two-hybrid assay. FIG. 6B shows GST-pulldowns of myc-tagged Fe65 expressed in COS cells with wild-type and mutant GST-Tip60 proteins. FIGS. 6C-F show immunofluorescence localization of tagged HA-Fe65 and myc-tagged Tip60 co-transfected into HeLa cells. FIG. 6C shows an overview of two adjacent cells; FIGS. 6D-F display individual and merged labeling patterns. Calibration bars=10 μ M.

[0015] FIG. 7A shows the results of transactivation of Gal4-Tip60 in COS cells co-transfected with full length or mutant Fe65 proteins and/or wild-type or mutant APP proteins. FIG. 7B shows the domain structure of Gal4-Tip60.

[0016] FIG. 8 provides a model for nuclear signaling mediated by the γ -cleavage product of APP in which APP is cleaved by α -, β - and γ -secretases, releasing the 47 residue cytoplasmic tail plus 10-12 hydrophobic residues from the TMR. The cytoplasmic tail complexed with Fe65 is translocated to the nucleus and interacts with Tip60 to regulate transcription.

DETAILED DESCRIPTION OF THE INVENTION

[0017] Proteolytic processing of APP produces, inter alia, amyloid- β peptide that contributes to the pathogenesis of Alzheimer's disease, and a C-terminal cytoplasmic tail of APP of unknown function until the present invention. In accordance with the present invention, it has been discovered that the C-terminal cytoplasmic tail of APP engages in nuclear signaling. In particular, it has been discovered that the released cytoplasmic tail of APP forms a functional complex with the nuclear protein Fe65 and the histone acetyl-transferase Tip 60, and that the complex is a potent stimulator of transcription by heterologous DNA-binding domains. This understanding of the role of the cytoplasmic tail of APP in nuclear signaling has led to the development of an assay to identify agents that affect the cleavage of APP. Such agents are useful as candidate therapeutics for the treatment of Alzheimer's disease, and as models for rational drug design.

[0018] In one embodiment, the present invention provides a method of identifying an agent that affects the cleavage of APP comprising contacting a cell, wherein the cell contains APP modified in the C-terminal cytoplasmic tail to allow detection of nuclear localization, with a candidate agent and measuring nuclear localization of the C-terminal cytoplas-

mic cleavage product of APP in the presence and absence of a candidate agent. An agent that increases or decreases the nuclear localization of the cleavage product relative to nuclear localization in the absence of the agent is defined as an agent that affects cleavage of APP.

[0019] The term APP as used herein includes naturally occurring mammalian APP and also APP that has been modified, for example in a way to facilitate measurement of nuclear localization of a cleavage product. Naturally occurring human APP is a 695 amino acid protein, in which the C-terminal 47 residues are designated the cytoplasmic tail. The gene encoding APP, its splice variants, and resulting nucleotide and amino acid sequences are known in the art and disclosed for example by Kang et al. (1987) Nature 325, 733-736, Selkoe, supra; Bayer et al., supra; Haass et al., supra; and Price et al., supra, the disclosures of which are incorporated herein by reference.

[0020] Further, APP as defined herein may include other modifications such as insertions, deletions and substitutions provided that the functions of ability of the cytoplasmic tail or part thereof to be cleaved from the remainder of APP and translocated to the nucleus are retained.

[0021] The C-terminal cytoplasmic tail of APP is modified allow detection of nuclear localization. The modification may be in any region of the cytoplasmic tail. The modification may be at the C-terminal or N-terminal end of the tail, for example at the junction of the transmembrane and cytoplasmic domains. In one embodiment, the cytoplasmic tail of APP is modified to include the DNA binding domain and the activation domain of the same or different heterologous transcription factors. Heterologous as used herein means not derived from a gene encoding APP. In this embodiment, nuclear localization is measured by determining activation of transcription of an indicator gene that is under the transcriptional control of a binding site for the DNA binding domain. Transcription factors and their component DNA-binding and activation domains are well-known in the art.

[0022] In a preferred embodiment, the cytoplasmic tail is modified to include a heterologous DNA-binding domain such as the DNA-binding domain of the yeast transcription factor Gal4, or the bacterial LexA DNA binding domain. The Gal4 and LexA DNA binding domains are known in the art and disclosed for example by Giniger et al. (1985) Cell 40, 767-774 and Hurstel et al. (1986) EMBO J. 5, 793-798. The modification may further contain the transcriptional activation domain of Gal4, or another activator such as the viral VP16 activator, which is disclosed for example by Stringer et al. (1990) Nature 345, 783-786. In a preferred embodiment, the cytoplasmic tail of APP is modified to include Gal4 and VP16. A transcription factor module of Gal4-VP16 is described by Sadowski et al. (1988) Nature 335, 563-564. In an alternative embodiment, a DNA-binding domain but not an activation domain is included with the cytoplasmic tail and the mammalian nuclear multidomain protein, Fe65, is added as a co-factor to facilitate transcriptional activation. Fe65 is known in the art and disclosed for example by Duilio et al. (1991) Nucleic Acids Res. 19, 5269-5274. The term Fe65 as used herein includes modifications such as insertions, deletions and substitutions provided that the function of the ability of Fe65 to facilitate transcriptional activation

is maintained. For example, it has been demonstrated herein that the N-terminal third of Fe65 may be deleted without loss of this function.

[0023] Accordingly, the modification of the cytoplasmic tail may consist of a heterologous DNA-binding domain, or a module consisting of a DNA-binding domain and a transcriptional activation domain, which may be from the same or different sources.

[0024] The indicator gene is operably linked to a binding site for the DNA-binding protein. For example, the indicator gene may be provided in the form of a Gal4 or LexA dependent reporter plasmid containing an indicator gene such as luciferase or chloramphenicol acetyl transferase under the control of a Gal4 or LexA regulatory element, respectively, such as an upstream activating sequence. Translocation of the cytoplasmic tail of APP to the nucleus results in translocation of the transcription factor as well, resulting in activation of transcription of the marker gene. Accordingly, detection of the marker gene product provides an assay for nuclear localization of the cytoplasmic tail of APP, and hence measures cleavage of APP. Transcriptional activation assays are described by Fields et al. (1989) *Nature* 340, 245-246, the disclosure of which is incorporated herein by reference. Gal4 and LexA reporter plasmids are described by Lillie et al. (1989) *Nature* (London) 338, 38-44 and Hollenberg et al. (1995) *Mol. Cell. Biol.* 15:3813-3822.

[0025] Candidate agents that may be tested by the assays of the present invention include proteins, peptides, non-peptide small molecules, and any other source of therapeutic candidate agents. The agents may be naturally occurring or synthetic, and may be a single substance or a mixture. Screening may be performed in high throughput format using combinatorial libraries, expression libraries and the like. Agents identified as affecting APP cleavage may be subsequently tested for biological activity and used as therapeutics or as models for rational drug design.

[0026] Cells useful for the assays of the present invention include eukaryotic cells in which the cytoplasmic tail cleavage product of APP can be translocated to the nucleus. Suitable cells include, for example, insect and mammalian cells. Preferred cells include Schneider, PC12, COS, HeLa and HEK293 cells.

[0027] Cells containing APP may be cells stably or transiently transfected with a construct encoding APP as described above using methods known to those of ordinary skill in the art. Constructs containing chimeric genes comprising a promoter operably linked to nucleic acid encoding APP and modified to include the DNA-binding domain of a transcriptional activator, or a module comprising the DNA-binding domains and transcriptional activation domain of the same or different transcription factor, are constructed using well-known recombinant DNA methods. These constructs are co-transfected into cells with the corresponding reporter constructs described above. For cases in which the construct does not contain a transcriptional activation domain, cells are also co-transfected with vector comprising a nucleic acid encoding Fe65 operably linked to a promoter. The promoter may be constitutive or inducible.

[0028] The transfected cells are contacted with an agent to be tested for its ability to affect APP cleavage. A detectable increase or decrease in nuclear localization of the C-terminal

cytoplasmic tail, as measured by a change in transcriptional activation of the indicator gene, is indicative of an agent that affects cleavage of APP. The cells may be contacted with the candidate agent before expression of modified APP is induced from an inducible promoter.

[0029] In a particularly preferred embodiment of the present invention human APP is modified to include Gal4-VP16 within the cytoplasmic tail. In particular, Gal4-VP16 is inserted between residues 651 and 652 of APP. The modified APP is generated by means of a mammalian expression plasmid containing a chimeric gene encoding residues 1-651 of APP, Gal4, VP16, and residues 652-695 of APP (i.e. the cytoplasmic tail, APP ct) under the control of a promoter. The plasmid may further comprise regulatory sequences, linkers, and other elements to facilitate cloning, replication, transfection and expression. A cell comprising the modified APP is provided by transfecting a cell, preferably a mammalian cell, and most preferably a human cell, with the expression plasmid. The cell is cotransfected with a Gal4 reporter plasmid in which luciferase mRNA is driven by multiple copies of the Gal4 upstream activating sequence (UAS). When the modified APP is cleaved by γ -secretase, the cleavage product containing Gal4-VP16 enters the nucleus and transactivates transcription from the Gal4 reporter plasmid. Expression of luciferase is measured by standard assays, for example by measuring luciferase activity using a commercially available kit. Luciferase expression is a measure of transactivation, which is in turn a measure of APP cleavage.

[0030] The transfected cells are contacted with a candidate agent, and luciferase expression is measured in the presence and absence of the agent. An agent that increases or decreases luciferase expression is an agent that affects APP cleavage.

[0031] In another embodiment, the present invention provides a method of identifying an agent that affects the cleavage of APP comprising providing a cell, wherein the cell contains APP and a protein that interacts with the C-terminal cleavage product of APP in the nucleus to activate transcription, and wherein the protein is modified to allow detection of nuclear translocation of the C-terminal cytoplasmic cleavage product; contacting the cell with a candidate agent; and measuring nuclear localization of the C-terminal cytoplasmic cleavage product in the presence and absence of the agent. An agent that increases or decreases nuclear localization of the C-terminal cleavage product relative to nuclear localization in the absence of the cleavage product is defined as an agent that affects cleavage of APP.

[0032] In accordance with the present invention, it has been discovered that the C-terminal cytoplasmic cleavage product of APP may interact with one or more other proteins to activate transcription. Accordingly, cleavage of APP can be detected by modifying a protein that interacts, directly or indirectly, with the cleavage product. Direct interaction refers to proteins that form a complex with the cleavage product. Indirect action includes proteins that interact with other proteins that are targets of the cleavage product, and thereby includes, for example, proteins that interact with Fe65 and/or Tip60 in the regulation of transcription. A preferred protein is the histone acetyl-transferase Tip60. In a preferred embodiment of this method, a protein that

interacts with the C-terminal cleavage product of APP to activate transcription, for example Tip60 is modified to allow detection of nuclear localization, for example by fusion with the DNA binding domain of a transcriptional activator such as Gal4 or LexA. Nuclear localization is measured by determining activation of transcription of an indicator gene that is under the transcriptional control of a binding site for the DNA binding domain, as described hereinabove.

[0033] The nucleotide and amino acid sequences of Tip60 are known in the art and disclosed for example by Kamine et al. (1996) *Virology* 216, 357-366 and Ran et al. (2000) *Gene* 258, 141-146. The term Tip60 as used herein includes modifications such as insertions, deletions and substitutions provided that the ability of Tip60 to interact with the C-terminal cytoplasmic cleavage product of APP is maintained.

[0034] In a preferred embodiment of this method, the cells contain APP, Fe65 and Tip60 modified to contain the DNA binding domain of a transcriptional activator, preferably Gal4. Such cells may be obtained by co-transfection with plasmids containing nucleic acids encoding APP, Fe65, and modified Tip60. Plasmids may further comprise regulatory sequences, linkers and other elements to facilitate cloning, replication, transfection and expression. Cells are eukaryotic, including for example insect and mammalian, and preferably human. Cells are also co-transfected with the appropriate reporter plasmid as described above. Expression of the reporter gene is a measure of transactivation, which is in turn a measure of nuclear localization of the C-terminal cytoplasmic cleavage product of APP, and thus APP cleavage. Reporter gene expression is measured as described hereinabove.

[0035] In another embodiment the present invention provides vectors that contain nucleic acids encoding the modified APP. In a preferred embodiment, the vector comprises a nucleic acid encoding APP operably linked to a promoter wherein a nucleic acid module encoding a heterologous DNA binding domain of a transcription factor and a transcriptional activator of the same or a different transcription factor is contained within the portion of the nucleic acid that encodes the C-terminal cytoplasmic tail of APP. A module "within" the tail includes embodiments in which the module is at the 5'-end or 3'-end of the region encoding the cytoplasmic tail. In a preferred embodiment the module is Gal4-VP16. The vectors may further comprise regulatory sequences, linkers, and other elements to facilitate cloning, replication, transfection and expression.

[0036] The present invention further provides vectors that comprise a nucleic acid encoding APP operably linked to a promoter wherein a nucleic acid encoding a heterologous DNA binding domain of a transcription factor is contained with the portion of the nucleic acid that encodes the C-terminal cytoplasmic tail of APP. The DNA binding domain may be at the 5'-end or 3'-end of the region encoding the cytoplasmic tail. In a preferred embodiment the DNA binding domain is Gal4. The vectors may further comprise regulatory sequences, linkers, and other elements to facilitate cloning, replication, transfection and expression.

[0037] The present invention further provides vectors that comprise a nucleic acid encoding Tip60 and the DNA binding domain of a transcription factor wherein the nucleic

acid is operably linked to a promoter. In a preferred embodiment the DNA binding domain is Gal4. The vectors may further comprise regulatory sequences, linkers, and other elements to facilitate cloning, replication, transfection and expression.

[0038] The present invention further provides cells containing the foregoing vectors. The cells are eukaryotic, preferably mammalian, and most preferably human. Cells containing the vectors of the invention may be obtained by methods known in the art, and may be transiently or stably transfected. The cells may also further contain a corresponding reporter plasmid as described hereinabove.

[0039] In another embodiment, the present invention provides agents that affect cleavage of APP identified by the method of the present invention. Compositions comprising the agents are also provided. The compositions may comprise carriers and/or diluents such as solvents, dispersion media, antibacterial and antifungal agents, microcapsules, liposomes, cationic lipid carriers, isotonic and absorption delaying agents and the like, as well as supplementary active ingredients.

[0040] The present invention further provides kits useful for identifying an agent that affects cleavage of APP. The kits comprise a first compartment containing cells comprising a vector that encodes a modified APP of the invention. The cells may further contain a reporter plasmid. The kits may further comprise a second compartment containing a means for measuring expression of an indicator gene contained in the reporter plasmid.

[0041] In another embodiment, the kits comprise a first compartment containing a vector that encodes a modified APP of the invention. The kits may further comprise a second compartment containing a reporter plasmid, and may further comprise a third compartment containing cells suitable for transfection by the vector of the first compartment.

[0042] In a further embodiment, the kits comprise a first compartment containing a vector comprising a nucleic acid encoding APP operably linked to a promoter, a second compartment containing a vector comprising a nucleic acid encoding Fe65 operably linked to a promoter, and a third compartment containing a nucleic acid encoding a fusion protein comprising Tip60 and the DNA binding domain of a transcriptional activator, preferably Gal4.

[0043] The following nonlimiting examples serve to further illustrate the present invention.

EXAMPLE I

Materials and Methods

[0044] The following materials and methods were used in subsequent examples.

[0045] 1. Transactivation Assays

[0046] 1.1 General Design

[0047] PC12, COS, HeLa, and HEK293 cells were co-transfected at 50-80% confluency in 6-well plates using Eugene6 (Roche, Indianapolis, Ind.), and 3-4 plasmids (0.1-1.0 μ g DNA/well depending on cell types; see plasmid list below for description of all constructs). All transfections included a. Gal4 (pG5E1B-luc) or LexA (L8G5-luc) reporter

plasmids; b. constitutively expressed β -galactosidase expression plasmid (pCMV-LacZ) to control for transfection efficiency; and c. the Gal4- or LexA-fusion protein vectors. Cells were harvested 48 hr post-transfection in 0.2 ml/well reporter lysis buffer (Promega, Madison, Wis.), and their luciferase and β -galactosidase activities were determined with the Promega luciferase assay kit and the standard O-nitrophenyl -D-galacto-pyranoside method, respectively. The luciferase activity was standardized by the β -galactosidase activity to control for transfection efficiency and general effects on transcription, and in most experiments further normalized for the transactivation observed in cells expressing Gal4 or LexA alone. Values shown are averages of transactivation assays carried out in duplicate, and repeated at least three times for each cell type and constructs. Most constructs were assayed in three or four cell lines, but usually only representative results for one cell line are shown. To confirm expression of transfected proteins and secretase cleavage of the various APP constructs, transfected cells were also analyzed by immunoblotting using antibodies to the respective proteins and/or antibodies to the epitope tags attached to the proteins.

[0048] 1.2 Amounts of DNA Used

[0049] 1.2.1. APP-Gal4VP16 Assays

[0050] Plasmids used in the following amounts: a. pG5E1B-luc (HEK293 cells, HeLa cells, and COS cells=0.3-0.5 μ g DNA; PC12 cells=1.0 μ g); b. pCMV-LacZ (HEK293 cells, HeLa cells, and COS cells=0.05 μ g DNA; PC12 cells=0.5 μ g DNA); c. pMst (Gal4), pMst-GV-APP (APP-GV), pMst-GV (GV), pMst-GV-APPct (APPct-GV), pMst-APPct (APPct-Gal4), pMst-GV-APP* (APP*-GV), pMst-GV-APP γ * (APP γ *-GV), pMst-APPct* (APPct*-Gal4), pMst-GV-APP α (APP α -GV), pMst-GV-NRX (NRX-GV), pMst-GV-NA (NRXe-GV-APPc), pMst-GV-AN (APPe-GV-NRXc) (HEK293, HeLa, and COS cells=0.1-0.3 μ g DNA; PC12 cells=1.0 μ g DNA). d. pcDNA3.1-PS2D366A, pCMV-Mint1, or pCMV5-Fe65 (HEK293, HeLa, and COS cells=0.1-0.3 μ g DNA; PC12 cells=0.5 μ g DNA).

[0051] 1.2.2. APP-Gal4 and APP-LexA Assays With and Without Various Fe65 and Min1 Constructs

[0052] For transactivation by APP-Gal4 and APP-LexA constructs, cells were cotransfected with: a. pG5E1B-luc (Gal4 reporter plasmid) or pL8G5-luc (LexA-reporter plasmid) (HEK293 cells, HeLa cells, and COS cells=0.3 μ g DNA; PC12 cells=1.0 μ g); b. pCMV-LacZ (β -galactosidase control plasmid. HEK293 cells, HeLa cells, and COS cells=0.05 μ g DNA; PC12 cells=0.2-0.5 μ g DNA); c. pMst (Gal4), pMst-APP (APP-Gal4), pMst-APP* (APP*-Gal4), pMst-APP γ (APP γ -Gal4), pMst-APP γ * (APP γ *-Gal4), pMst-AN-APPc32 (APP-G-NRX-APPc32), pMst-AN (APPe-G-NRXc), pML (LexA), pML-APP (APP-LexA), pML-APP* (APP*-LexA), pML-APPct (APPct-LexA), pML-APPct* (APPct*-LexA) (HEK293 cells, HeLa cells, and COS cells=0.3-0.5 μ g DNA; PC12 cells=1.0-1.5 μ g); and d. pCMV-Mint1 (mint1) or pCMV5-Fe65 (Fe65) (HEK293 cells, HeLa cells, and COS cells=0.3-0.5 μ g DNA; PC12 cells=1.0-1.5 μ g) where indicated.

[0053] For transactivation assays of Fe65 mutants, cells were cotransfected with: a. pG5E1B-luc (Gal4 reporter plasmid) or pL8G5-luc (LexA-reporter plasmid); b. pCMV-

LacZ (B-galactosidase control plasmid); c. pMst (Gal4), pMst-APP (APP-Gal4), pML (LexA), or pML-APP (APP-LexA); and d. pCMV5-Fe65 (Fe65), pCMVMyc-Fe65 (128-711) (Fe65 (128-711)), pCMVMyc-Fe65 (242-711) (Fe65 (242-711)), pCMVMyc-Fe65 (287-711) (Fe65 (287-711)), pCMV5-Fe65 (1-553) (Fe65PTB2), pCMVMyc-Fe65APTb1 (Fe65APTb1), pCMV5-Fe65 mW1 (Fe65 mW1), pCMV5-Fe65 mW2 (Fe65 mW2), pCMV5-Fe65 mW3 (Fe65 mW3), pCMV5-Fe65 mW4 (Fe65 mW4), or pCMV5-Fe65 mW5 (Fe65 mW5) where indicated. a. and b.: amounts of DNA same as under 1.21. c. and d.:0.3-0.5 μ g DNA for COS, HeLa, and HEK293 cells; 1.0-1.5 μ g DNA for PC12 cells.

[0054] 1.2.3. Gal4-Tip60 Assays

[0055] For transactivation assays of Gal4-Tip60, COS and HEK293 cells were cotransfected with: a. pG5E1B-luc (Gal4 reporter plasmid, 0.3 μ g DNA); b. pCMV-LacZ (β -galactosidase control plasmid, 0.05 μ g DNA); c. pMst (Gal4); pM-Tip60 (rat Gal4-Tip60 M residues 63-454); pM-Tip60* (mutant rat Gal4-Tip60 P residues 63-454); pM-hTip60 (full-length wild type human Gal4-Tip60 β); or pM-hTip60* (full-length mutant human Gal4-Tip60 β) (0.4 μ g DNA) d. pCMV5-Fe65 (Fe65), pCMVMyc-Fe65 (242-711) (Fe65 (242-711)), pCMV5-Fe65 (1-553) (Fe65)PTB2, or pCMV5-Fe65 mW4 (Fe65 mW4) (0.3 μ g DNA) where indicated; and e. pCMV5-APP (human APP695) or pCMV5-APP* (mutant human APP695) (0.3 μ g DNA). All transfections contained one of the plasmids listed in a-c, whereas d and e were variable.

[0056] 2. Yeast Two-Hybrid Screens for APP- and Fe65-Binding Proteins

[0057] 2.1 APP Screens

[0058] Bait: pBTM116-APP

[0059] Yeast strain: L40

[0060] Library: P8 rat brain library constructed in prey vector pVP16-3.

[0061] Screening condition: 250 ml mid-log phase yeast harboring the bait vector pBTM116-APP were transformed with 125 μ g of P8 rat brain library plasmids, and plated on CSM-Trp-Leu-His plates supplemented with 5 mM 3-amino-1,2,4-triazole. 2x10⁶ total transformants were obtained. Around 250 positives appeared after 3 days incubation at 30° C. 80 positives were recovered and identified by sequencing or Southern blotting. Among them, 72 are Fe65 and one is Fe65-like protein 2 (Fe65LP2).

Summary of Fe65 prey clones that were sequenced

Prey Clones	Insert Size (kb)	Residues	Extra Residues
P29 ^a	3.1	1-711	43
P50, P64, P65, P69	2.9	1-711	43
P42	3.0	48-711	0
P60 ^a	2.6	128-711	0
P6 ^a , P7, P18	1.9	242-711	8
P57	2.3	242-711	50
P19, P25 ^b	1.0	242-?	21
P15, P27 ^a	1.6	287-711	0
P21	1.5	301-711	0
P4, P22	1.4	313-711	0

-continued

Summary of Fe65 prey clones that were sequenced			
Prey Clones	Insert Size (kb)	Residues	Extra Residues
P9, P16, P17	1.9	321-711	0
P39	1.4	339-711	0

^aP29, P60, P6, P27 were used to generate pCMV5-Fe65, pCMVMyc-Fe65(128-711), pCMVMycFe65(242-711), and pCMVMyc-Fe65(287-711), respectively.
^bP25 insert (1.0 kb) was used as Southern blotting probe to identify the rest of the recovered prey clones.

[0062] 2.2 Fe65 Screens

- [0063] Bait: pLexN-Fe65 (287-711)
- [0064] Yeast strain: L40
- [0065] Library: P8 rat brain library constructed in prey vector pVP16-3.
- [0066] Screening condition: 250 ml mid-log phase yeast harboring the bait vector pLexN-Fe65 (287-711) were transformed with 125 μ g of P8 rat brain library plasmids, and plated on CSM-Trp-Leu-His plates supplemented with 25 mM 3-amino-1,2,4-

triazole. 7×10^7 total transformants were obtained, and 300 positives selected after 3 days incubation at 30° C.

[0067] Summary of prey clones that were sequenced: Tip60 β (residues 63-454)=8 clones; APLP1=9 clones.

[0068] 3. Quantitative Yeast Two-Hybrid Assays

[0069] For quantitative measurements of yeast two-hybrid interactions, various bait and prey plasmids were co-transformed into yeast strain L40 and plated on CSM-Trp-Leu (Bio101) plates. Single colonies from the CSM-Trp-Leu plates were cultured in CSM-Trp-Leu liquid medium after 3 days, and re-inoculated into YPAD medium at OD₆₀₀=0.3 on the following day. When the yeast cultures reached mid-log phase (OD₆₀₀=0.6-1.0), cells were collected by centrifugation, washed in Z buffer (16.1 g/l Na₂HPO₄·7H₂O, 5.5 g/l NaH₂PO₄·H₂O, 0.75 g/l KCl, 0.246 g/l MgSO₄·7H₂O pH 7.0), and resuspended in 100 μ l Z buffer. Cells were lysed by three freeze-thawing cycles, 0.8 ml reaction solution (0.5 g/l ONPG and 0.024% β -mercaptoethanol in Z buffer) was added, and reactions were stopped with 0.4 ml 1M Na₂CO₃ at the appropriate time points depending on color development. After centrifugation (14,000 $\times$ g for 10 min), OD₄₂₀ was measured, and the relative β -galactosidase activity was calculated as 1000 $\times$ OD₄₂₀/(min $\times$ vol. yeast in ml $\times$ OD₆₀₀).

[0070] 3.1

APP & Fe65							
#	Bait	Prey	OD ₆₀₀	Time (min)	OD ₄₂₀	β -gal unit	Mean S.D.
1	APPct	P29(Fe65 1-711)	0.899	15	5.025	372.6363	423.8138 48.30529
			0.764	15	4.93	430.192	
			0.685	15	4.815	468.6131	
2	APPct	Fe65mW5	0.657	15	4.895	496.7022	449.4991 71.701238
			0.68	15	4.945	484.8039	
			0.822	15	4.525	366.9911	
3	APPct	vector	0.813	15	0.004	0.328003	0.370065 0.0425321
			0.903	15	0.005	0.36914	
			0.807	15	0.005	0.413052	
4	APPct*	P29(Fe65 1-711)	0.776	15	0.005	0.429553	0.506697 0.0674611
			0.601	15	0.005	0.554631	
			0.622	15	0.005	0.535906	
5	APPct*	Fe65mW5	0.631	15	0.004	0.42261	0.44523 0.0467628
			0.644	15	0.004	0.414079	
			0.668	15	0.005	0.499002	

a. APPct and P29(Fe65 1-711) are bait and prey clones described in the section on the Yeast two-hybrid screen for APP-binding proteins.
b. APPct* was expressed from pBTM116-APP*, which was generated by QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) to mutate 684NPTY to 684NATA with pBTM116-APP as the template.
c. Fe65mW5 was expressed from pVP16-3-Fe65mW5, which encodes full-length rat Fe65 (1-711 residues) with Y270A, Y271A, W272A, W281F, and P284A mutations.

[0071] 3.2

Fe65 & LBP-1c							
#	Bait	Prey	OD ₆₀₀	Time (min)	OD ₄₂₀	β -gal unit	Mean S.D.
1	Fe65(287-711)	LBP-1c	0.747	60	0.037	0.825524	0.772376 0.1010779
			0.737	60	0.029	0.655812	
			0.678	60	0.034	0.835792	
2	Fe65(287-531)	LBP-1c	0.686	60	0.167	4.057337	4.438847 0.3744772
			0.704	60	0.203	4.805871	
			0.625	60	0.167	4.453333	

-continued

Fe65 & LBP-1c							
#	Bait	Prey	OD ₆₀₀	Time (min)	OD ₄₂₀	Ξ-gal unit	S.D.
3	Fe65(287–711)	vector	0.709	60	0.006	0.141044	0.15965
			0.703	60	0.008	0.189663	
			0.787	60	0.007	0.148242	
4	Fe65(287–531)	vector	0.725	60	0.022	0.505747	0.5543
			0.681	60	0.026	0.636319	
			0.672	60	0.021	0.520833	
5	Lamin	LBP-1c	0.626	60	0.017	0.452609	0.721477
			0.819	60	0.03	0.610501	
			0.454	60	0.03	1.101322	

a. Fe65(287–711) and Fe65(287–531) were expressed from pLexN-Fe65(287–711) and pLexN-Fe65(287–531), respectively. Lamin was expressed from pBTM116-Lamin.
b. LBP-1c was expressed from pVP16-3-LBP-1c, which encodes full-length human LBP-1c (1–450 residues). References: Z. Bing, S. A. G. Reddy, Y. Ren, J. Qin, and W. S. L. Liao (1999) J. Biol. Chem. 274, 24649–24656. N. Zambrano, G. Minopoli, P. de Candia, and T. Russo (1998) J. Biol. Chem. 273, 20128–20133.

[0072] 3.3

Fe65 & hTip60							
#	Bait	Prey	OD ₆₀₀	Time (min)	OD ₄₂₀	Ξ-gal unit	S.D.
1	Fe65(287–711)	hTip60	0.806	15	4.905	405.7072	431.7927
			0.818	15	4.975	405.4605	
			0.665	15	4.83	484.2105	
2	Fe65(287–531)	hTip60	0.695	15	4.895	373.6211	391.1748
			0.774	15	4.055	349.2679	
			0.682	15	4.61	450.6354	
3	Fe65(287–711)	vector	0.709	60	0.006	0.141044	0.15965
			0.703	60	0.008	0.189663	
			0.787	60	0.007	0.148242	
4	Fe65(287–531)	vector	0.725	60	0.022	0.505747	0.5543
			0.681	60	0.026	0.636319	
			0.672	60	0.021	0.520833	
5	Lamin	hTip60	0.594	15	0.011	1.234568	1.751572
			0.635	15	0.017	1.784777	
			0.507	15	0.017	2.235371	

a. Fe65(287–711) and Fe65(287–531) were expressed from spLexN-Fe65(287–711) and pLexN-Fe65(287–531), respectively. Lamin was expressed from pBTM116-Lamin.
b. hTip60 was expressed from pVP16-3-hTip60 which encodes full-length human Tip60β (residues 1–461). Reference: Qitao Ran and Olivia M. Pereira-Smith (2000) Gene 258, 141–146.

[0073] 4. Plasmid List

[0074] 4.1 Standard Plasmids

[0075] pCMV-LacZ: Transfection control plasmid encoding bacterial β-galactosidase under control of the CMV promoter.

[0076] pG5E1B-luc: Gal4 reporter plasmid (Lillie, J. W., and M. R. Green. 1989. Transcription activation by the adenovirus E1a protein. Nature (London) 338:39-44) in which luciferase mRNA is driven by five copies of Gal4 UAS.

[0077] pL8G5-luc: LexA reporter plasmid in which luciferase mRNA is driven by eight copies of the LexA binding site and five copies of Gal UAS. (Hollenberg, S. M., Sternglanz, R., Cheng, P. F., and Weintraub H. 1995. Identification of a new family of tissue-specific basic Helix-Loop-Helix proteins with a two-hybrid system. Mol. Cell. Biol. 15:3813-3822).

[0078] pMst: Gal4 expression vector driven by the SV40 promoter derived from pM (Clontech, Palo Alto, Calif.) by mutating the stop codon before the Gal4 DNA-binding domain.

[0079] pMst-GV: Gal4 VP16 (GV) expression vector generated by cloning the VP16 activation domain (residues 413-490) into the EcoRI/BamHI sites of pMst (linker sequence between Gal4 and VP16: QLTVSPEFAPPTD).

[0080] pML: LexA expression vector generated by replacing the NheI/EcoRI fragment of pM (Clontech) with the PCR amplified LexA-coding sequence.

[0081] 4.2 APP Plasmids

[0082] 4.2.1. Mammalian Expression Plasmids

[0083] pMst-GV-APPct, encodes APPct-GV generated by cloning the cytoplasmic tail of human APP695 (APPct,

residues 652-695) into the BamHI/SalI sites of pMst-GV (linker sequence between GV and APPct=DEYGGGIP-PGQYTSI).

[0084] pMst-GV-APPct*, encodes APPct*-GV with point mutations in the cytoplasmic tail of APP (residues 684-687; wild type sequence=NPTY; mutant sequence=NATA). Generated by QuickChange site-directed mutagenesis (Stratagene) with pMst-GV-APPct as template.

[0085] pMst-GV-APP encodes APP-GV. Generated by cloning a PCR fragment encoding residues 1-651 of human APP695 into the NheI site of pMst-GV-APPct (linker sequence between APPE and GV=MLKKKPLASSRMKLLS).

[0086] pMst-GV-APP*, encodes APP*-GV with point mutations in the cytoplasmic tail of APP (residues 684-687; wild type sequence=NPTY; mutant sequence=NATA). Generated by QuickChange site-directed mutagenesis (Stratagene) with pMst-GV-APP as the template.

[0087] pMst-GV-APP γ , encodes APP γ -GV containing an N-terminal methionine followed by residues 639-651 of human APP695, Gal4-VP16, and residues 652-695 of APP695. Obtained by inserting the PCR-amplified residues 639-651 into the BglII/NheI sites of pMst-GV-APPct (linker sequence between TMR of APP and Gal4-MLKKKPLASSRMKLLS).

[0088] pMst-GV-APP γ *, encodes APP γ *-GV corresponding to APP γ -GV with the mutation in the NPTY sequence. Generated by QuickChange site-directed mutagenesis (Stratagene) with pMst-GV-APP γ as the template.

[0089] pMst-APPct, encodes APPct-Gal4. Generated by cloning the cytoplasmic tail of human APP695 (APPct, residues 652-695) into the BamHI/SalI sites of pMst (linker sequence between Gal4 and APPct=QLTVSPEFPGIP-PGQYTSI).

[0090] pMst-APPct*, encodes APPct*-Gal4 corresponding to APPct-Gal4 with the NPTY mutation. Generated by cloning the mutant cytoplasmic tail from pMst-GV-APPct* into the BamHI/SalI sites of pMst.

[0091] pMst-APP, encodes APP-Gal4. Generated by cloning a PCR fragment containing the extracellular and transmembrane region of human APP695 (APPE, residues 1-651) into the NheI site of pMst-APPct (linker sequence between APPE and Gal4=MLKKKPLASSRMKLLS).

[0092] pMst-APP*, encodes APP*-Gal4. Obtained as pMst-APP, but cloned into the NheI site of pMst-APPct*.

[0093] pMst-APP γ , encodes APP γ -Gal4. Generated by cloning a PCR fragment coding for a methionine and residues 639-651 of human APP695 into the BglII/NheI sites of pMst-APPct (linker sequence between TMR and Gal4=MLKKKPLASSRMKLLS).

[0094] pMst-APP γ *, encodes APP γ *-Gal4. Obtained as pMst-APP γ , but cloned into pMst-APPct*.

[0095] pMst-GV-NRX, encodes NRX-GV. Generated by sequential cloning of PCR fragments coding for the cytoplasmic tail of rat neurexin I (β NRXct, residues 414-468) and the extracellular and transmembrane regions (NRXe, residues 1-417) into the BamHI/SalI and the NheI sites, respectively, of pMst-GV (linker sequences between NRXe

and Gal4=MYKYRTLASSRMKLLS; between VP16 and NRXct=DEYGGGIPPGYKYRN).

[0096] pMst-GV-NA, encodes NRXe-GV-APPc. Generated by cloning a PCR fragment corresponding to NRXe into the NheI site of pMst-GV-APPct (linker sequence between NRXe and Gal4=MYKYRTLASSRMKLLS).

[0097] pMst-GV-AN, encodes APPE-GV-NRXc. Generated by sequential cloning of PCR fragments corresponding to NRXe and APPE into the BamHI/SalI and the NheI sites, respectively, of pMst-GV (linker sequences between APPE and Gal4=MLKKKPLASSRMKLLS; between VP16 and NRXct=DEYGGGIPPGYKYRN).

[0098] pMst-AN encodes APPE-G-NRXc. Generated as pMst-GV-AN, but into pMst (linker sequences between APPE and Gal4=MLKKKPLASSRMKLLS, and between Gal4 and NRXct=QLTVSPEFPGIPPGYKYRN).

[0099] pMst-AN-APPc32, encodes APP-G-NRX-APPc32. Generated from pMst-AN by inserting a PCR fragment encoding the C-terminal 32 residues of human APP695 (APPc32) into the rat neurexin I cytoplasmic tail (NRXct) between residue 425 and 427, with V426 deleted during the cloning (linker sequences between APPc32 and NRXct=EGSYHIDDAAVT, and between APPc32 and NRXct=EQMQNIDESRN).

[0100] pML-APPct, encodes APPct-LexA. Generated by replacing the Gal4 sequence (the NheI/EcoRI fragment) in pMst-APPct with the LexA-sequence (linker sequence between LexA and APPct=NGDWLEFPGIPPGYTSI).

[0101] pML-APPct*, encodes APPct*-LexA. Generated as pML-APPct in pMst-APPct*.

[0102] pML-APP, encodes APP-LexA. Generated by cloning the extracellular and transmembrane region of human APP695 (APPE, residues 1-651) into the NheI site of pML-APPct (linker sequence between APPE and LexA=MLKKKPLAKMKALT).

[0103] pML-APP*, encodes APP*-LexA. Generated as pML-APP with pML-APPct*.

[0104] pCMV5-APP, encodes full-length human APP695 inserted into the blunted-EcoRI/XbaI sites of pCMV5.

[0105] pCMV5-APP*, encodes full length human APP695 containing point mutations in the cytoplasmic NPTY sequence. Generated by QuickChange site-directed mutagenesis (Stratagene) with pCMV5-APP.

[0106] 4.2.2. Yeast Two-Hybrid Plasmids

[0107] pBTM116-APP, encodes residues 648-695 of human APP695 cloned into the BamHI/SalI sites of the yeast two-hybrid bait vector pBTM116 using a PCR fragment.

[0108] pBTM116-APP*=pBTM116-APP in which the codons encoding the NPTY sequence in the APP cytoplasmic tail were mutated to NATA using QuickChange site-directed mutagenesis (Stratagene).

[0109] 4.3 Fe65 Plasmids

[0110] 4.3.1. Mammalian Expression Plasmids

[0111] pCMV5-Fe65: encodes full-length rat Fe65 (711 residues). Constructed by sub-cloning the 3 kb SalI fragment from the yeast two-hybrid prey clone #P29 into the SalI site of pCMV5.

[0112] pCMVMyc-Fe65 (128-711): encodes residues 128-711 of Fe65. Generated by cloning the blunt-ended fragment from the rat Fe65 cDNA into the blunted EcoRI site in pCMVMyc.

[0113] pCMVMyc-Fe65 (242-711): encodes residues 242-711 of Fe65. Generated by cloning the blunt-ended . . . fragment from the rat Fe65 cDNA into the blunted EcoRI site in pCMVMyc.

[0114] pCMVMyc-Fe65 (287-711): encodes residues 287-711 of Fe65. Generated by cloning the blunt-ended . . . fragment from the rat Fe65 cDNA into the blunted EcoRI site in pCMVMyc.

[0115] pCMV5-Fe65 (1-553): encodes Fe65 PTB2 which lacks residues 554-711 of Fe65. Generated by introducing a stop codon into pCMV5-Fe65 after residue 553 with the QuickChange site directed mutagenesis kit (Stratagene).

[0116] pCMVMyc-Fe65 ΔPTB1: encodes Fe65 ΔPTB1 which lacks residues 314-440. Generated by sequentially cloning the PCR fragments encoding residues 441-711 and residues 1-313 of rat Fe65 into the ClaI and MluI sites, respectively, of pCMVMyc.

[0117] pCMV5-Fe65mW1: encodes Fe65 mW1 point mutant in carrying substitutions W281F and P284A. Generated by QuickChange site directed mutagenesis (Stratagene) with pCMV5-Fe65 as template.

[0118] pCMV5-Fe65 mW2: encodes Fe65 mW2 point mutant in carrying substitution W260F. Generated by QuickChange site directed mutagenesis (Stratagene) with pCMV5-Fe65 as template.

[0119] pCMV5-Fe65 mW3: encodes Fe65 mW3 point mutant in carrying substitutions W260F, W28SF and P284A. Generated by QuickChange site directed mutagenesis (Stratagene) with pCMV5-Fe65mW1 as template.

[0120] pCMV5-Fe65 mW4: encodes Fe65 mW4 point mutant in carrying substitutions Y270A, Y271A, and W272A. The insert (2.1 kb) can be cut out by HindIII+Sall double digestion.

[0121] pCMV5-Fe65 mW5: encodes Fe65 mW4 point mutant in carrying substitutions Y270A, Y271A, W272A, W281F, and P284A. The insert (2.1 kb) can be cut out by HindIII+Sall double digestion.

[0122] pcDNA3.1-N-HA-Fe65: encodes full-length rat Fe65 preceded by a hemagglutinin (HA) epitope. Obtained by subcloning the rat Fe65 cDNA into the blunted-EcoRI/XbaI sites of pcDNA3.1-N-HA.

[0123] 4.3.2. Yeast Two-Hybrid Plasmids

[0124] pLexN-Fe65 (287-711), encodes residues 287-711 of Fe65 in the Sall/blunted-PstI sites in pLexN.

[0125] pLexN-Fe65 (287-531), encodes residues 287-531 of Fe65 in the BamHI/blunted-Sall sites in pLexN. Insert (740 bp) can be cut out by BamHI+PstI double digestion.

[0126] pVP16-3-Fe65 mW5, encodes the mW5 mutant of Fe65 (see pCMV vectors above). Was generated by cloning the blunt-ended 2.1 kb HindIII/XbaI fragment from pCMV5-Fe65 mW5 into the blunt-ended-NotI/XbaI sites of the yeast prey vector pVP16-3. The insert can be cut out by Sall.

[0127] 4.4 Tip60 Plasmids

[0128] 4.4.1. pCMV Expression Plasmids

[0129] pCMVMyc-Tip60 (63-454), encodes residues 63-454 of rat Tip60β. Generated by cloning the 1.3 kb EcoRI fragment from yeast two-hybrid prey clone #B36 into the EcoRI site of pCMVMyc.

[0130] pCMVMyc-Tip60 (63-454)*, encodes residues 63-454 of rat Tip60p with a mutation in residues 257-260 (sequences: wildtype=NKSY; mutant=NASA). Generated by QuickChange site directed mutagenesis kit (Stratagene) with pCMVMyc-Tip60 (63-454) as template.

[0131] pM-Tip60 encodes rat Tip60 residues 63-454 preceded by the Gal4 DNA-binding domain. Generated by subcloning the 1.3 kb BamHI/XbaI fragment from prey clone #36 into the BamHI/XbaI sites of pM.

[0132] pM-Tip60* encodes the same protein as pM-Tip60 with the inactivating mutation in residues 257-260. Generated by cloning the 1.3 kb BamHI/XbaI fragment from pVP16-3-Tip60* into the BamHI/XbaI sites of pM.

[0133] pCMVMyc-hTip60 encodes myc-tagged full-length human Tip6013. Obtained by subcloning the insert of EST IMAGE clone 2901054 into the MluI/XbaI sites of pCMVMyc.

[0134] pM-hTip60 encodes full-length human Tip60 preceded by the Gal4-DNA binding domain. Obtained by cloning the blunt-ended 1.6 kb EcoRI/NotI fragment from EST clone 2901054 into the blunt-ended EcoRI site of pM. Insert can be cut out by Sall.

[0135] pM-hTip60*, same as pM-hTip60 but with the inactivating mutation in residues 257-260. Generated by QuickChange site directed mutagenesis (Stratagene) with pM-hTip60 as template.

[0136] 4.5 Yeast Two-Hybrid Plasmids

[0137] Prey clone #B36 in pVP16-3; identified in yeast two-hybrid screens with pLexN-Fe65 (287-711) as the bait in a P8 rat brain library. B36 encodes rat Tip60B corresponding to residues 63-454 of human Tip60, with a single amino acid change between human and rat sequences.

[0138] pVP16-3-Tip60* encoding mutant rat Tip60 with the inactivating mutation in residues 257-260 (sequences: wildtype=NKSY; mutant=NASA). Generated by cloning the EcoRI fragment from pCMVMyc-Tip60 (63-454)* into the EcoRI site of yeast prey vector pVP16-3.

[0139] pVP16-3-hTip60, full-length human Tip60 cloned into the EcoRI/NotI sites of pVP16-3.

[0140] 4.6 GST-Fusion Protein Plasmids

[0141] pGEX-KG-Tip60 (63-454), residues 63-454 Tip60β fused to GST. Generated by cloning the 1.3 kb EcoRI fragment (1.3 kb) of the yeast two-hybrid prey clone B36 into the EcoRI site of pGEX-KG.

[0142] pGEX-KG-Tip60 (63-454)*, encodes residues 63-454 Tip60β fused to GST. Generated as pGEX-KG-Tip60 (63-454), but from pVP16-3-Tip60 (63-454)*.

[0143] 5. Miscellaneous Plasmids**[0144]** 5.1 pCMV Expression Plasmids

[0145] pCMV5-Mint1: rat Mint1 cloned into the EcoRI site of pCMV5 (Okamoto, M. and Südhof, T. C. (1997) *J. Biol. Chem.* 272, 31459-31464.)

[0146] pCS2+MT-SEF: Myc-tagged full-length human LBP-1c (1-450 residues) was expressed from control of the CMV promoter (gift from Dr. W. S. L. Liao, University of Texas MD Anderson Cancer Center, Houston Tex.; reference: Z. Bing, S. A. G. Reddy, Y. Ren, J. Qin, and W. S. L. Liao (1999) *J. Biol. Chem.* 274, 24649-24656.)

[0147] pcDNA3.1-PS2D366A (kind gift of Dr. C. Haass, Munich): encodes a dominant negative mutant of human presenilin 2 in pcDNA3.1.

[0148] 5.2 Yeast Two-Hybrid Plasmids

[0149] pVP16-3-LBP-1c, encodes full-length human LBP-1c. Generated by cloning the blunted 1.4 kb XhoI fragment from pCS2+MT-SEF into the blunted NotI site of the yeast prey vector pVP16-3. Insert can be cut out by XhoI.

[0150] 6. Transfections, Transactivation Assays and Yeast Two Hybrid Screens

[0151] PC12, COS, HeLa, and HEK293 cells were cotransfected at 50-80% confluency in 6-well plates using Eugene6 (Roche), and 3-5 plasmids (0.1-1.0 μ g DNA/well depending on cell types; see plasmid list hereinabove for description of all constructs). All transfections included a. Gal4 (pG5E1B-luc) or LexA (pL8G5-luc) reporter plasmids; b. constitutively expressed β -galactosidase expression plasmid (pCMV-LacZ) to control for transfection efficiency; and c. the Gal4- or LexA-fusion protein vectors. Cells were harvested 48 hr post-transfection in 0.2 ml/well reporter lysis buffer (Promega), and their luciferase and β -galactosidase activities were determined with the Promega luciferase assay kit and the O-nitrophenyl-D-galacto-pyranoside method, respectively. The luciferase activity was standardized by the β -galactosidase activity to control for transfection efficiency and general effects on transcription, and in most experiments normalized for the transactivation observed in cells expressing Gal4 or LexA alone. Values shown are averages of transactivation assays carried out in duplicate, and repeated at least three times for each cell type and constructs. Most constructs were assayed in three or four cell lines, but usually only representative results for one cell line are shown. To confirm expression of transfected proteins and secretase cleavage of the various APP constructs, transfected cells were also analyzed by immunoblotting using antibodies to the respective proteins and/or antibodies to the epitope tags attached to the proteins.

[0152] For assays of the transactivation by Gal4-VP16 constructs, cells were cotransfected with a. pG5E1B-luc (Gal4 reporter plasmid); b. pCMV-LacZ (13-galactosidase control plasmid); c. pMst (Gal4), pMst-GV-APP (APP-GV), pMst-GV (GV), pMst-GV-APPct (APPct-GV), pMst-APPct (APPct-Gal4), pMst-GV-APP* (APP*-GV), pMst-GV-APPct* (APPct*-GV), pMst-APPct* (APPct*-Gal4), pMst-GV-APP γ (APP γ -GV), pMst-GV-NRX (NRX-GV), pMst-GV-NA (NRX-GV-APPe), or pMst-GV-AN; and d. pcDNA3.1-PS2D366A (kind gift of Dr. C. Haass, Munich), pCMV-Mint1; or pCMV5-Fe65 where indicated.

[0153] A yeast two-hybrid cDNA library in pVP16-3 was screened with pBTM116-APP encoding the cytoplasmic tail of human APP₆₉₅ as described (Vojtek et al. (1993) *Cell* 74, 205-214; Okamoto et al. (1997) *J. Biol. Chem.* 272: 31459-31464). Of 80 positive clones, 72 encoded Fe65 and one Fe65-like protein. The full-length rat Fe65 sequence has been submitted to GenBank (Acc.#AF333983). Interactions of all proteins including mutants of Fe65 were quantified using liquid β -galactosidase assays on yeast strains harboring various bait and prey clones (see Example 1).

[0154] For transactivation by APP-Gal4 and APP-LexA constructs, cells were cotransfected with: a. pG5E1B-luc (Gal4 reporter plasmid) or pL8G5-luc (LexA-reporter plasmid); b. pCMV-LacZ (β -galactosidase control plasmid); c. pMst (Gal4), pMst-APP (APP-Gal4), pMst-APP* (APP*-Gal4), pMst-APP γ (APP γ -Gal4), pMst-APP γ * (APP γ *-Gal4), pMst-AN-APPc32 (APP-G-NRX-APPc32), pMst-AN (APPe-G-NRXc), pML (LexA), pML-APP (APP-LexA), pML-APP* (APP*-LexA), pML-APPct (APPct-LexA), pML-APPct* (APPct*-LexA); and d. pCMV-Mint1 (mint1) or pCMV5-Fe65 (Fe65) where indicated. Analyses were performed as described above.

[0155] COS7 cells were transfected in 100 mm dishes using DEAE-dextran or Eugene6 (Roche) with single or combinations of expression vectors encoding wild-type and mutant APPct-Gal4, APP γ -Gal4, and APP-Gal4, myc-tagged or HA-tagged wild type or mutant Fe65, and wild type and mutant Tip60 (see above for a description of the expression vectors), and harvested 72 hr after transfection. For the immunoblotting experiments (**FIG. 3**), cell extracts were immunoblotted with polyclonal antibodies to the C-terminus of APP (U955) or to Fe65, and with monoclonal antibodies to Gal4 (Clontech) or to the myc- or HA-epitope (Santa Cruz). For the immunoprecipitation experiments (**FIG. 5**), cells were washed twice with cold PBS, harvested in 1 ml lysis buffer (50 mM HEPES-NaOH pH 7.5, 150 mM NaCl, 10% glycerol, 1% IGEPAL CA-630, 1.5 mM MgCl₂, 1 mM EGTA, 1 mM DTT, 0.1 g/L PMSF, 10 mg/L Leupeptin, 10 mg/L aprotinin, 1 mg/L pepstatin A), and passed through a 28 gauge needle 5x. Cell extracts were clarified by centrifugation at 20,800xg for 10 min. The supernatants (~1 ml) were incubated with 10 μ l of a polyclonal antibody raised against the C-terminus of APP (U955) or monoclonal antibodies to myc-tag (Santa Cruz) for 2 hr at 4° C., 60 μ l of a 50% slurry of protein A- or protein G-Sepharose (Pharmacia) were added, and the beads were incubated with the reactions for 1 hr at 4° C. on a rotator and then collected by centrifugation. Beads were washed 3x with lysis buffer, resuspended in 0.1 ml SDS-PAGE sample buffer, and 20 μ l of the protein solutions were resolved on 10% SDS-PAGE, and detected by immunoblotting with antibodies to APP, Gal4, or the myc-epitope.

[0156] For transactivation assays of Fe65 mutants, cells were cotransfected with: a. pG5E1B-luc (Gal4 reporter plasmid) or pL8G5-luc (LexA-reporter plasmid); b. pCMV-LacZ (B3-galactosidase control plasmid); c. pMst (Gal4), pMst-APP (APP-Gal4), pML (LexA), or pML-APP (APP-LexA); and d. pCMV5-Fe65 (Fe65), pCMVMyc-Fe65(128-711) (Fe65 (128-711)), pCMVMyc-Fe65 (242-711) (Fe65 (242-711)), pCMVMyc-Fe65 (287-711) (Fe65 (287-711)), pCMV5-Fe65 (1-553) (Fe65 PTB2), pCMVMyc-Fe65APT1 (Fe65APT1), pCMV5-Fe65 mW1 (Fe65 mW1), pCMV5-Fe65 mW2 (Fe65 mW2), pCMV5-Fe65 mW3 (Fe65 mW3), pCMV5-Fe65 mW4 (Fe65 mW4), or

pCMV5-Fe65 mW5 (Fe65 mW5) where indicated. Analyses were performed as described above, and plasmids are described above.

[0157] Yeast two-hybrid screens were carried out with a fragment from rat Fe65 (residues 287-711) as described above. Out of 100 clones analyzed, 9 clones encoded APLP1, and 8 clones Tip60p (residues 63-454 of the insert-minus splice β -variant; submitted to GenBank with Acc.#AF333984). The domains of Fe65 that bind to the cytoplasmic tail of APP or to Tip60 were studied by quantitative yeast two-hybrid assays which demonstrated that the first PTB domain of Fe65 is necessary and sufficient for binding to Tip60, and the second PTB domain for binding to APP. For Tip60, both the partial rat cDNA and the full-length human cDNA were analyzed (see **FIG. 6A**).

[0158] GST-pulldowns were performed essentially as described by Hata et al. (1993) *Nature* 366, 347-351 using purified wild type and mutant rat GST-Tip60 and Fe65 expressed by transfection in COS cells. Extracts from transfected COS cells were preabsorbed with 10 μ g GST on glutathione agarose for 2 hr at 4° C., and then incubated for 4 hrs at 4° C. with 10 μ g of GST-Tip60, GST-Tip60*, or GST bound to glutathione agarose. Beads were washed 5 $\times$ lysis buffer, resuspended in 80 PI SDS-PAGE sample buffer, and 20 μ l were analyzed by SDS-PAGE and immunoblotting using antibodies to Fe65 and to the myc epitope. Co-immunoprecipitation experiments of Fe65, APP, and wild-type and mutant rat Tip60 were performed as described above using COS cells co-transfected with the appropriate vectors.

[0159] HeLa cells plated on cover glass in a 12-well plate were transfected with pcDNA3.1-N-HA-Fe65 and pCMV-Myc-hTip60 (0.25 μ g for each plasmid) using Fugene6 (Roche). Two days after transfection, cells were washed twice with PBS, fixed (3.7% formaldehyde for 10 min at room temperature), and blocked and permeabilized in PBS containing 3% BSA, 0.1% IGEPAL CA-630 for 20 min. Cells were then incubated with anti-HA monoclonal antibody (BAbCO Berkeley antibody company) and anti-Myc polyclonal antibody (Upstate Biotechnology) for 1 hr (1:200 dilution in blocking buffer), washed with PBS 3 $\times$, and treated with Rhodamine-goat-anti-mouse and FITC-goat-anti-rabbit antibodies (Chemicom) for 1 hr (1:500 dilution in blocking buffer). After 3 washes with PBS and one wash with water, cells were mounted and observed with a confocal microscope.

[0160] For transactivation assays of Gal4Tip60, COS and HEK293 cells were cotransfected with: a. pG5E1B-luc (Gal4 reporter plasmid); b. pCMV-LacZ (β -galactosidase control plasmid); c. pMst (Gal4); pM-Tip60 (rat Gal4-Tip60 residues 63-454); pM-Tip60* (mutant rat Gal4-Tip60, residues 63-454); pM-hTip60 (full-length wild type human Gal4-Tip60 β); or pM-hTip60* (full-length mutant human Gal4-Tip60 β) d. pCMV5-Fe65 (Fe65), pCMVMyc-Fe65 (242-711) (Fe65 (242-711)), pCMV5-Fe65 (1-553) (Fe65 PTB2), or pCMV5-Fe65 mW4 (Fe65 mW4) where indicated; and e. pCMV5-APP (human APP695) or pCMV5-APP* (mutant human APP695). All transfections contained one of the plasmids listed in a-c, whereas d and e were variable. Analyses were performed as described above.

EXAMPLE 2

[0161] Nuclear signaling by the cytoplasmic γ -cleavage product of APP fused to Gal4-VP16. Cleavage of APP produces a C-terminal fragment composed of half of the TMR (10-12 residues) and the cytoplasmic tail (47 residues) (See, e.g. Selkoe (1998) *Trends Cell Biol.* 8, 447-453). A transcription factor was engineered into the cytoplasmic tail of APP. The inserted transcription factor was Gal4-VP16 which is composed of a yeast DNA-binding protein (Gal4) fused to a powerful viral activator (VP16) (Sadowski et al. (1988) *Nature* 335, 563-564). When inserted in the cytoplasmic tail of APP, Gal4-VP16 can only act as a transcription factor if APP is cleaved by γ -secretase, and if the resulting product enters the nucleus. Gal4-VP16 was inserted into full-length APP695 at the cytoplasmic boundary of the TMR, the resulting APP-Gal4-VP16 fusion protein was transfected into a variety of cell lines (PC12, HEK293, COS, or HeLa cells), and transactivation of transcription from a co-transfected Gal4-dependent reporter plasmid encoding luciferase was measured. Isolated Gal4-VP16 (without APP) was employed as a positive control, and Gal4 alone (without VP16 and APP) as a negative control. In all experiments, cells were co-transfected with a constitutive β -galactosidase expression plasmid in order to control for transfection efficiency, and verified protein expression by immunoblotting. Transfections and analyses were performed as described in Example 1.

[0162] Full-length APP-Gal4-VP16 (APP-GV) transactivated Gal4-dependent transcription much stronger than Gal4 alone in all cell types tested (~500-2,000 fold activation depending on cell type). Surprisingly, full-length APP-Gal4-VP16 was as powerful in activating Gal4-dependent transcription as free Gal4-VP16 (GV) without APP (**FIG. 1A#1-3**). Immunoblotting revealed that the transfected proteins were expressed well, and that APP-Gal4-VP16 was partly cleaved by α - or β - and γ -secretases in the cells, resulting in stable C-terminal fragments which could be detected by antibodies to Gal4 and to the cytoplasmic tail of APP. A chimeric protein in which Gal4-VP16 was only fused to the cytoplasmic tail of APP without the TMR and extracellular sequences of APP (APPct-GV) was more potent in transactivation than full-length APP-Gal4-VP16, or even Gal4-VP16 alone (~3,000 vs. ~1,000 fold activation; **FIG. 1A#5**). In contrast, the cytoplasmic APP tail containing only Gal4 without VP16 (APPct-Gal4) was only slightly more active than Gal4 alone (<5 fold activation; **FIG. 1A#8**). Together these results show that the cytoplasmic tail of APP released by γ -cleavage is competent to enter the nucleus, and may partly activate transcription. Similar results were obtained in all cells tested, and thus are not a unique property of a particular cell line.

[0163] To rule out the possibility that transactivation by APP-Gal4-VP16 in transfected cells may be caused by non-specific proteolysis of the APP-Gal4-VP16 fusion protein instead of γ -cleavage, the γ -cleavage product of APP with an inserted Gal4-VP 16 module in the cytoplasmic tail (APP γ -GV) was directly expressed, and its ability to activate Gal4-dependent transcription was measured (**FIG. 1A#6**). Similar to the cytoplasmic tail fragment of APP, the isolated γ -cleavage product was more active in transcription than full-length APP-Gal4-VP16 or Gal4-VP16 alone, confirming that the hydrophobic residues in the γ -cleavage product do not inhibit transactivation.

EXAMPLE 3

[0164] Sequence-specificity of transactivation mediated by APP-Gal4-VP16. The cytoplasmic tail of APP contains a conserved NPTY sequence that constitutes a binding site for the PTB-domains of at least three proteins, Fe65, Mints/X11s, and Disabled (Fiore et al. (1995) *J. Biol. Chem.* 270, 30853-30856; McLoughlin et al. *FEBS Lett.* 397, 197-200; Borg et al. (1996) *Mol. Cell. Biol.* 16, 6229-6241). Binding of these proteins to APP could contribute to the transcriptional activation mediated by APP-Gal4-VP16 by influencing γ -cleavage of APP, or by participating in nuclear translocation. This possibility was examined by mutating the NPTY sequence in the cytoplasmic tail of APP to NATA. Transactivation assays showed that in all cell types tested, the NPTY-mutants of Gal4-VP16 fusion proteins were as potent as wild type proteins in activating transcription (**FIG. 1A#4, 7 & 9**), suggesting that the NPTY motif and its binding proteins are not essential for transactivation by the Gal4-VP16 module inserted into APP. In agreement with this conclusion, co-transfection of Fe65 did not cause a major change in transactivation by APP-Gal4-VP16.

[0165] The specificity of transactivation by Gal4-VP16 inserted into a membrane protein was examined by introducing Gal4-VP16 into the cytoplasmic tail of neurexin 1 β as a control protein that is also expressed on the neuronal cell-surface but is not known to be processed by proteolytic cleavage (Ushkaryov et al. (1992) *Science* 257, 50-56). Neurexin 1 β -Gal4-VP16 (NRX-GV) did not exhibit transactivation in contrast to APP-Gal4-VP16 (**FIG. 1B, #3 & 10**), suggesting that not all cell-surface Gal4-VP16-fusion proteins are competent for transactivation. In these experiments, a dominant negative mutant of presenilin 2 (Steiner et al. (1999) *J. Biol. Chem.* 274, 28669-28673) was co-transfected with the Gal4-VP16-fusion proteins to test if presenilins are involved. As expected, transactivation by full-length APP-Gal4-VP16 was inhibited by the presenilin 2 mutant, whereas the small amount of residual transactivation observed with neurexin 1 β -Gal4-VP16 was insensitive to presenilin 2 (**FIG. 1B, #3 & 10**).

[0166] To determine which APP sequences enable the inserted Gal4-VP16 to activate transcription, chimeric proteins containing different combinations of the extracellular and intracellular sequences of APP and of neurexin 1 β were produced. When extracellular sequences and TMR of APP were fused to Gal4-VP16 and to the cytoplasmic tail of neurexin 1 β (APPe-GV-NRXc), potent transactivation was observed. In contrast, the reverse fusion protein of the extracellular domain and TMR of neurexin 1 β with the cytoplasmic tail of APP (NRXc-GV-APPe) was inactive (**FIG. 1B, #11 and 12**). Again, presenilin 2 inhibited the chimeric protein containing the extracellular domain of APP but had no effect on the residual transactivation observed with the protein containing the extracellular domain of neurexin 1, supporting the notion that specific APP-sequences are required for transactivation as studied by this assay.

EXAMPLE 4

[0167] Fe65 binding to the cytoplasmic tail of APP stimulates transcription. To identify co-factors that may be involved in nuclear signaling with the cytoplasmic tail of APP, yeast two-hybrid screens for proteins that bind to the

cytoplasmic tail of APP were performed as described in Example 1. Similar to previous screens (26-31), Fe65 was the major interacting protein identified, although it was isolated at an unexpectedly high frequency (90% of all clones).

[0168] A further assay was performed to determine whether Fe65 represents a co-factor for APP in nuclear signaling. Without Fe65, APP-Gal4 activated Gal4-dependent transcription only weakly (<10 fold). By contrast, co-expression of Fe65 with APP-Gal4 powerfully stimulated transcription (200-2,000 fold depending on the cell type). This was observed in all cell lines tested (PC12, HEK293, COS, or HeLa cells) (**FIGS. 2A-2C**). As a control, co-expression of mint 1/X11 which also binds to the cytoplasmic tail of APP (See, e.g., Fiore et al. (1995) *J. Biol. Chem.* 270, 30853-30856), had no major effect on transactivation under these conditions. Neither Fe65 nor mint1/X11 changed the transcription of the control 13-galactosidase plasmid co-transfected into all cells.

[0169] To examine whether Fe65 still stimulates transactivation when the Fe65-binding site in the cytoplasmic tail of APP (the NPTY sequence) is mutated, yeast two hybrid assays and co-immunoprecipitations were performed as described in Example 1. Replacing the NPTY sequence with NATA abolished Fe65 binding as shown by yeast two-hybrid assays and co-immunoprecipitations (see also **FIG. 5** below). In agreement with a direct role for Fe65 binding in stimulating transactivation by the cytoplasmic tail of APP, the same mutation also abolished the Fe65-dependent stimulation of transcription (**FIG. 2A**).

[0170] The hypothesis that Fe65 binds to the cytoplasmic γ -cleavage product of APP to activate Gal4-dependent transcription in the nucleus was confirmed by the observation that Fe65 powerfully stimulated transactivation by a "pre-cleaved" APP-Gal4 fragment corresponding to the γ -cleavage product (200-2,000 fold stimulation of transactivation depending on cell type; **FIG. 2B**). The effect of Fe65 depended on the intact Fe65-binding site in the cytoplasmic tail of APP (**FIG. 2B**). The relative activity of APP-Gal4 co-transfected with Fe65 compares well to that of the potent Gal4-VP16 fusion protein, suggesting that Fe65 is a powerful transcriptional activator. Further evidence for the notion that Fe65 needs to bind to the cytoplasmic APP tail in order to stimulate transcription was obtained with series of chimeric APP/neurexin constructs (**FIG. 2C**). When the cytoplasmic tail of APP-Gal4 was replaced with the cytoplasmic tail of neurexin 1 β , Fe65 did not stimulate transactivation. However, when the 32 amino acid Fe65-binding site from the cytoplasmic tail of APP was transplanted into the middle of the neurexin cytoplasmic tail, powerful stimulation of transcription (>200 fold over Gal4) was observed (**FIG. 2C**).

[0171] To ensure that APP-Gal4 is indeed cleaved at the γ -secretase site in the transfected cells, the size of the APP-Gal4 cleavage products was examined. COS cells were transfected with Gal4-fusion proteins of full-length APP (as test protein), and of the cytoplasmic tail and the γ -cleavage product of APP (as size standards to identify the correct cleavage product) as described above. Immunoblotting of the transfected cells with antibodies to the cytoplasmic tail of APP and to Gal4 detected two major APP cleavage products (**FIG. 3**). A fragment that was bigger than the

γ -cleavage product, tentatively identified as the α - or β -secretase product, and a fragment that of precisely the same size as the γ -cleavage product but slightly larger than the cytoplasmic tail protein alone (**FIG. 3**) were detected. No artifactual cleavage at the boundary of the TMR and the inserted Gal4 protein was detected, while the γ -secretase cleavage product was abundantly produced. Furthermore, analyses of cells co-transfected with Fe65 revealed that Fe65 had no apparent effect on the production of the α - and γ -cleavage products.

EXAMPLE 5

[0172] Fe65 stimulates transactivation independent of the DNA binding protein. Gal4 contains an intrinsic nuclear localization signal (Silver et al. (1984) *Proc. Natl. Acad. Sci. USA* 81, 5951-5955) and theoretically could cause non-specific transcriptional activation that could be unrelated to the normal functions of these proteins. To exclude Gal4-specific artifacts, APP was fused to the bacterial LexA DNA-binding protein (Smith et al. (1988) *EMBO J.* 7, 3975-3982), and measured Fe65-dependent transactivation with a LexA-dependent luciferase reporter as described in Example 3 (**FIG. 4**). In the absence of Fe65, APP-LexA did not mediate significant transactivation (**FIG. 4B**). However, co-transfection of Fe65 strongly stimulated transactivation (40-100 fold). The effect of Fe65 was less pronounced with LexA than with Gal4, possibly because the bacterial LexA DNA-binding domain acting on a bacterial promoter sequence is less optimal for mammalian transcription. Similar to the Gal4 system, however, Fe65 stimulated transactivation both with full-length APP and with the isolated cytoplasmic tail, and stimulation depended on the intact Fe65-binding site in the cytoplasmic tail of APP.

EXAMPLE 6

[0173] The WW-domain and both PTB-domains of Fe65 are required for transactivation. Fe65 is a multidomain protein that contains a negatively charged N-terminal sequence with no homology to other proteins, a central WW-domain, and two C-terminal PTB-domains (Ermeikova et al. (1998) *Adv. Exp. Med. Biol.* 446, 161-180; McLoughlin et al. (1998) *Biochem. Soc. Trans.* 26, 497-500). The WW-domain of Fe65 interacts with the cytoskeletal adaptor protein mena (Ermeikova et al. (1997) *J. Biol. Chem.* 272, 32869-32877). The second PTB-domain of Fe65 (PTB2) binds to the cytoplasmic tails of APP and other cell-surface proteins containing NPxY motifs (Fiore et al. (1995) *J. Biol. Chem.* 270: 30853-30856). In addition to these cytoplasmic activities, Fe65 has been implicated in nuclear functions. Fe65 is partly localized to the nucleus, its first PTB-domain (PTB1) binds to the transcription factor CP2/LSF/LBP1, and its negatively charged N-terminal sequences stimulates Gal4-dependent transcription (Duilio et al. (1991) *Nucleic Acids Res.* 19, 5269-5274). The foregoing data establish that the γ -cleavage product of APP forms a complex with Fe65 that transactivates a heterologous promoter, suggesting that the APP/Fe65 complex functions as a transcriptional activator.

[0174] To investigate how the APP/Fe65 complex activates transcription, a series of Fe65 deletion mutants were constructed and their ability to stimulate transactivation was determined as described in Example 1. Both APP-Gal4 and APP-LexA were used to ensure that the effects observed

were not peculiar to a particular DNA-binding protein (**FIG. 5**). The WW-domain and both PTB-domains of Fe65 were found to be essential for activating transcription. By contrast, deletion of the N-terminal third of Fe65 with the acidic region suspected of activating transcription had no effect on transactivation (**FIGS. 5A and 5B**). The results indicate that in addition to the binding of the second PTB domain of Fe65 to the cytoplasmic tail of APP, the WW-domain and the first PTB domain also interact with target molecules in order for Fe65 to stimulate transcription.

[0175] The Fe65 deletion mutants suggest that Fe65 is a true adaptor protein in transcriptional regulation. To exclude possible artifacts induced by the deletions, immunoblotting was performed to confirm that all of the transfected proteins were stably expressed, and not prematurely degraded. Immunoprecipitations from COS cells which co-express wild type or mutant Fe65 and APP-Gal4 showed that deletion of the first PTB-domain in Fe65 does not impair its ability to bind to the cytoplasmic tail of APP as long as that tail contains a wild type NPTY sequence (**FIGS. 5C and 5D**). Finally, point mutations in the WW-domain were used instead of a large deletion to assess the need for the WW domain in the stimulation of transactivation. Substitution of one of the conserved tryptophan residues of the WW-domain had no effect, while replacement of the central YYW motif with alanine residues abolished the Fe65-dependent stimulation of transcription (**FIGS. 5A and 5B**). Again, immunoblotting confirmed that all mutants were stably expressed, and none of the Fe65 proteins influenced basal transcription from the co-transfected control plasmids. Together these experiments show that all three canonical domains of Fe65 are required to activate transcription in a complex with the cytoplasmic tail of APP, establishing that Fe65 is a genuine adaptor protein which links multiple components into a single active complex.

EXAMPLE 7

[0176] Fe65 binds to the histone acetyl transferase Tip60. As an adaptor protein, Fe65 presumably directly or indirectly interacts with transcription factors when it activates transcription. A candidate for such a binding protein is the transcription factor LBP/CP2/LSF which interacts with the first PTB-domain of Fe65 (Duilio et al. (1991) *Nucleic Acids Res.* 19, 5269-5274). However, only a weak interaction between LBP/CP2/LSF and Fe65 was observed in quantitative yeast two-hybrid assays, and no change in the amount of transactivation was detected when LBP/CP2/LSF was co-transfected with Fe65 and APP-Gal4. Other Fe65-interacting proteins were searched for using yeast two-hybrid screens as described in Example 1. A single prey clone that strongly bound to the first PTB-domain of Fe65, and that contains almost the entire coding sequence for Tip60, was identified. Tip60 is a histone acetyl transferase that is expressed in two alternatively spliced forms (Tip60 α and β), interacts with multiple transcription factors, and is part of a large complex in the nucleus (Kamine et al. (1996) *Virology* 216, 357-366). Quantitative yeast two-hybrid assays, GST-pulldown studies, and co-immunoprecipitation experiments confirmed a strong interaction of Fe65 with both the partial rat Tip60 β obtained in the yeast two-hybrid screens, and with full-length human Tip60 β (**FIGS. 6A and 6B**). Furthermore, immunofluorescence analyses of transfected cells

showed that Fe65 and Tip60 β colocalize in the nucleus in a speckled pattern, suggesting that they function as a complex (FIGS. 6C-6F).

[0177] PTB-domains usually bind to NPxY target sequences, although variant binding sequences have also been observed (Zwahlen et al. (2000) EMBO J. 19, 15005-15015). In a search for a possible PTB-domain target sequence in Tip60, a single motif was detected that is remotely similar to the NPxY sequence (NKSY; residues 257-260). Mapping of the NKSY sequence onto the three-dimensional structure of Esa1, a yeast histone acetyl-transferase whose three-dimensional structure has been solved (Yan et al. (2000) *Mol. Cell* 6, 1195-1205), suggests that the NKSY motif in Tip60 is located on a surface loop of a conserved domain, and thus accessible for a binding partner. To test if the Fe65 PTB1-domain binds to this site, the Tip60 NKSY sequence was mutated into NASA, and the ability of mutant Tip60 to bind to Fe65 was tested. No binding was observed for the mutant as measured either by quantitative yeast two-hybrid assays or GST-pulldowns (FIGS. 6A and 6B), suggesting that the first PTB-domain of Fe65 binds to the NKSY sequence in Tip60.

EXAMPLE 8

[0178] Binding of the APP/Fe65 complex to Tip60 mediates transactivation. To test if Fe65 enhances transactivation by binding to the Tip60 complex, a Gal4-Tip60 fusion protein was constructed, and the effects of Fe65 and APP on Gal4-dependent transactivation mediated by the Gal4-Tip60 fusion protein were examined (FIG. 7). Gal4-Tip60 alone was unable to support significant Gal4-dependent transcription (no activation over Gal4 alone). Co-expression of either

Fe65 or APP individually with Gal4-Tip60 did not enhance transactivation. However, when Gal4-Tip60 was co-expressed with both Fe65 and APP, transactivation was stimulated dramatically (p100 fold; FIG. 7). Mutant APP that is unable to bind to Fe65 (APP*) was largely inactive (~100 fold enhancement of transactivation). Furthermore, no potentiation of transactivation was observed when Fe65 and APP were co-expressed with mutant Gal4-Tip60 (Gal4-Tip60*) that is unable to bind to Fe65, or with Gal4 only. Together these data show that the cytoplasmic tail of APP has a direct active role in stimulating transactivation, and that it collaborates with Fe65 in enhancing transcription by Gal4-Tip60.

[0179] In experiments described above (FIG. 5), it was found that all three canonical Fe65 domains (the WW domain and the two PTB domains) are required for Fe65 to potentiate transactivation by Gal4- and LexA-APP proteins. In order to test if the same applies for the Fe65- and APP-dependent transactivation by Gal4-Tip60, a series of Fe65 mutants was examined in this assay (FIG. 7). The N-terminal sequence of Fe65 was not needed for potentiating Gal4-Tip60 dependent transactivation, whereas the second PTB-domain that binds to APP was essential. The WW domain of Fe65 was also found to be indispensable.

[0180] The foregoing data provide a model for the function of APP and its homologs whereby proteolytic cleavage of APP releases the cytoplasmic tail to activate a nuclear signal in transcription (FIG. 8).

[0181] All references cited herein are incorporated herein in their entirety.

SEQUENCE LISTING

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<210> SEQ ID NO 2

<211> LENGTH: 2520

<212> TYPE: DNA

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 2

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<210> SEQ ID NO 3

<211> LENGTH: 1980

<212> TYPE: DNA

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 3

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gccatgaacg ccgcctactc gggctatgtc tacacgcacc ggctcttcca ccgcgcgag 540
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aacgggatta acccagaaga cctgagccag aaggagtaca gcgacctgct caacaccag 1920
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<210> SEQ ID NO 4
<211> LENGTH: 1224
<212> TYPE: DNA
<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 4

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ctactctcag acaaaactcc ctccaaaaac gtgcgcatga tgcaggccca ggaagcagta	180
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<210> SEQ ID NO 5
<211> LENGTH: 2253
<212> TYPE: DNA
<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 5

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atccgctatt gtcctgagga tgacagctac ctggagggca tggactgtaa tggggaggag	360
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<210> SEQ ID NO 6

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<212> TYPE: DNA

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 6

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 <212> TYPE: DNA
 <213> ORGANISM: rattus norvegicus
 <400> SEQUENCE: 7

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ggtcctatcc cccatgacca ggagcctgag aatgaggata cagaactgcc tctggagagc	120
tatgtaccca caggcctgga gctaggcact ctgagaccag acagccccac gcctgaggaa	180
caggagtgcc acaaccatag ccctgatggg gactccagct ctgactatgt gaacaacacg	240
tctgaggagg aggactatga cgagggcctc cctgaggagg aggaaggtgt cacctactac	300
atccgctatt gtcctgagga tgacagctac ctggagggca tggactgtaa tggggaggag	360
tacctagccc atggtgcaca tcctgtggac actgatgagt gtcaggaggc ggtagaggac	420
tggacggact cagtgggtcc tcatactcat agccacgggg ctgaaaacag ccaagagtat	480
ccagacagcc acctgcctat ccagaggat gaccctactg tcctggaggc ccatgaccag	540
gaagaagatg gccactactg tcccagcaag gagagctacc aggactatta tccccagag	600
accaatggga acacgggtgg cgcttctccc tatcgcatga ggcgtgggga tggggaccta	660
gaggagcagg aggaagacat cgaccagata gtggtgaga tcaagatgag cctgagcatg	720
accagtatta ccagtgccag tgaggccagc cctgagcaca tgcttgagct ggaccctggg	780
gactccactg aggcctgttc acccagtgc actggcgtg gaccagtag gcaagaagcg	840
aggcccaagt cgctgaacct tccccctgag gttaagcact ccggagaccc ccaaagagga	900
ctcaagacca agaccaggac cccagaggag aggcctaaagt ggcctcaaga gcaggtttgc	960
aatggcttgg aacagccgag gaagcagcag cgctctgac tcaatggacc cactgacaat	1020
aacaacatcc cagagacaaa gaaggtggcc tcgtttccaa gctttgtagc tgttcaggg	1080
ccctgtgagc cagaagacct catcgatggc atcatctttg cagccaaacta cctgggctcc	1140
accagctgc tctctgagcg caaccctcc aaaaacatcc gaatgatgca agctcaagaa	1200
gctgtcagca gggtaagag gatgcagaag gctgctaaga tcaagaaaa agcgaattct	1260
gagggtgatg ctgacacact gacagaagta gacctcttca tttctaccca gaggatcaaa	1320
gtcttaaaag ctgacacaca ggaaccatg atggaccatg ccttgcgac catctctac	1380
attgcagaca ttgggaacat cgtggttctg atggccaggc gccgcatgcc caggtcagcc	1440
tctcaggact gcatcgagac cagcctggg gccaggaag ggaagaagca gtacaagatg	1500
atctgtcacg tgttcgagtc agaggatgcc cagctgatag ccagtcfaat tgggcaggcc	1560
ttcagtgtgg cctaccagga gtctctgagg gccaacggca tcaaccctga ggacctgagc	1620
cagaaggaat acagtgatat cataaatacc caggagatgt ataatgatga ccttatccac	1680
ttctcaaaact cggagaactg caaggagctc tag	1713

<210> SEQ ID NO 8

<211> LENGTH: 1227

<212> TYPE: DNA

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 8

atggagcaga agctgatcag cgaggaggac ctgaacggaa ttcagatctg gtaccctgt	60
gagccagaag acctcatcga tggcatcato tttgcagcca actacctggg ctccaccag	120
ctgctctctg agcgcaacct ctccaaaaac atccgaatga tgcaagctca agaagctgtc	180
agcagggtca agaggatgca gaaggctgct aagatcaaga aaaaagcgaa ttctgaggg	240
gatgctcaga cactgacaga agtagacctc ttcatcttca ccagaggat caaagtctta	300
aacgctgaca cacaggaaac catgatggac catgccttgc gcaccatctc ctacattgca	360

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gacattggga acatcgtggt tctgatggcc aggcgccgca tgcccaggtc agcctctcag	420
gactgcatcg agaccacgcc tggggcccag gaagggaaga agcagtacaa gatgatctgt	480
cacgtgttcg agtcagagga tgcccagctg atagcccagt caattgggca ggccttcagt	540
gtggcctacc aggagtctct gagggccaac ggcatcaacc ctgaggacct gagccagaag	600
gaatacagtg atatacataa taccaggag atgtataatg atgaccttat ccacttctca	660
aactcggaga actgcaagga gctgcagctg gagaagcaca agggtgagat ttgggtgtg	720
gtggtcgtgg agtcaggctg gggctccatc ctgccactg tgatcctggc gaacatgatg	780
aacggcggcc cagcagctcg ctgggggaag ctgagcattg gcgaccagat catgtccatc	840
aatggcacca gcctggtggg gctgcccctc gctacctgcc agggatatcat caagggcctg	900
aagaaccaa cagaggtaaa gctcaacatc gtcagctgtc cccagtcac cacagtctc	960
atcaaacgtc cagatctcaa gtaccagctg ggtttcagcg tgcaaatgg aatcatttgc	1020
agcctcatga gagggggtat tgcagagcga ggtggtgtcc gagtcggcca ccgtatcatc	1080
gagatcaacg gacagagtgt ggtagccaca gccacgaga agatagtcca ggctctgtct	1140
aactcagttg gagagattca catgaagacc atgcctgcag ccatgttcag gctcctcaca	1200
ggccaggaga caccgctgta catctag	1227

<210> SEQ ID NO 9
<211> LENGTH: 839
<212> TYPE: PRT
<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 9

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

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Glu	Ala	Arg	Asp	Gly	Leu	Arg	Leu	Tyr	Glu	Arg	Glu	Arg	Asp	Glu	Ala	210	215	220
Ala	Ala	Tyr	Arg	Gln	Glu	Ala	Leu	Gly	Ala	Arg	Leu	His	His	Tyr	Asp	225	230	235
Glu	Arg	Ser	Asp	Gly	Glu	Ser	Asp	Ser	Pro	Glu	Lys	Glu	Ala	Glu	Phe	245	250	255
Ala	Pro	Tyr	Pro	Arg	Met	Asp	Ser	Tyr	Glu	Gln	Glu	Glu	Asp	Ile	Asp	260	265	270
Gln	Ile	Val	Ala	Glu	Val	Lys	Gln	Ser	Met	Ser	Ser	Gln	Ser	Leu	Asp	275	280	285
Lys	Ala	Ala	Glu	Asp	Met	Pro	Glu	Ala	Glu	Gln	Asp	Leu	Glu	Arg	Ala	290	295	300
Pro	Thr	Pro	Gly	Gly	Gly	His	Pro	Asp	Ser	Pro	Gly	Leu	Pro	Ala	Pro	305	310	315
Ala	Gly	Gln	Gln	Gln	Arg	Val	Val	Gly	Thr	Pro	Gly	Gly	Ser	Glu	Val	325	330	335
Gly	Gln	Arg	Tyr	Ser	Lys	Glu	Lys	Arg	Asp	Ala	Ile	Ser	Leu	Ala	Ile	340	345	350
Lys	Asp	Ile	Lys	Glu	Ala	Ile	Glu	Glu	Val	Lys	Thr	Arg	Thr	Ile	Arg	355	360	365
Ser	Pro	Tyr	Thr	Pro	Asp	Glu	Pro	Lys	Glu	Pro	Ile	Trp	Val	Met	Arg	370	375	380
Gln	Asp	Ile	Ser	Pro	Thr	Arg	Asp	Cys	Asp	Asp	Gln	Arg	Pro	Val	Asp	385	390	395
Gly	Asp	Ser	Pro	Ser	Pro	Gly	Ser	Ser	Ser	Pro	Leu	Gly	Ala	Glu	Ser	405	410	415
Ser	Ile	Thr	Pro	Leu	His	Pro	Gly	Asp	Pro	Thr	Glu	Ala	Ser	Thr	Asn	420	425	430
Lys	Glu	Ser	Arg	Lys	Ser	Leu	Ala	Ser	Phe	Pro	Thr	Tyr	Val	Glu	Val	435	440	445
Pro	Gly	Pro	Cys	Asp	Pro	Glu	Asp	Leu	Ile	Asp	Gly	Ile	Ile	Phe	Ala	450	455	460
Ala	Asn	Tyr	Leu	Gly	Ser	Thr	Gln	Leu	Leu	Ser	Asp	Lys	Thr	Pro	Ser	465	470	475
Lys	Asn	Val	Arg	Met	Met	Gln	Ala	Gln	Glu	Ala	Val	Ser	Arg	Ile	Lys	485	490	495
Thr	Ala	Gln	Lys	Leu	Ala	Lys	Ser	Arg	Lys	Lys	Ala	Pro	Glu	Gly	Glu	500	505	510
Ser	Gln	Pro	Met	Thr	Glu	Val	Asp	Leu	Phe	Ile	Ser	Thr	Gln	Arg	Ile	515	520	525
Lys	Val	Leu	Asn	Ala	Asp	Thr	Gln	Glu	Pro	Met	Met	Asp	His	Pro	Leu	530	535	540
Arg	Thr	Ile	Ser	Tyr	Ile	Ala	Asp	Ile	Gly	Asn	Ile	Val	Val	Leu	Met	545	550	555
Ala	Arg	Arg	Arg	Met	Pro	Arg	Ser	Asn	Ser	Gln	Glu	Asn	Val	Glu	Ala	565	570	575
Ser	His	Pro	Ser	Gln	Asp	Ala	Lys	Arg	Gln	Tyr	Lys	Met	Ile	Cys	His	580	585	590
Val	Phe	Glu	Ser	Glu	Asp	Ala	Gln	Leu	Ile	Ala	Gln	Ser	Ile	Gly	Gln	595	600	605

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Ala Phe Ser Val Ala Tyr Gln Glu Phe Leu Arg Ala Asn Gly Ile Asn
 610 615 620
 Pro Glu Asp Leu Ser Gln Lys Glu Tyr Ser Asp Leu Leu Asn Thr Gln
 625 630 635 640
 Asp Met Tyr Asn Asp Asp Leu Ile His Phe Ser Lys Ser Glu Asn Cys
 645 650 655
 Lys Asp Val Phe Ile Glu Lys Gln Lys Gly Glu Ile Leu Gly Val Val
 660 665 670
 Ile Val Glu Ser Gly Trp Gly Ser Ile Leu Pro Thr Val Ile Ile Ala
 675 680 685
 Asn Met Met His Gly Gly Pro Ala Glu Lys Ser Gly Lys Leu Asn Ile
 690 695 700
 Gly Asp Gln Ile Met Ser Ile Asn Gly Thr Ser Leu Val Gly Leu Pro
 705 710 715 720
 Leu Ser Thr Cys Gln Ser Ile Ile Lys Gly Leu Lys Asn Gln Ser Arg
 725 730 735
 Val Lys Leu Asn Ile Val Arg Cys Pro Pro Val Thr Thr Val Leu Ile
 740 745 750
 Arg Arg Pro Asp Leu Arg Tyr Gln Leu Gly Phe Ser Val Gln Asn Gly
 755 760 765
 Ile Ile Cys Ser Leu Met Arg Gly Gly Ile Ala Glu Arg Gly Gly Val
 770 775 780
 Arg Val Gly His Arg Ile Ile Glu Ile Asn Gly Gln Ser Val Val Ala
 785 790 795 800
 Thr Pro His Glu Lys Ile Val His Ile Leu Ser Asn Ala Val Gly Glu
 805 810 815
 Ile His Met Lys Thr Met Pro Ala Ala Met Tyr Arg Leu Leu Thr Ala
 820 825 830
 Gln Glu Gln Pro Val Tyr Ile
 835

<210> SEQ ID NO 10

<211> LENGTH: 839

<212> TYPE: PRT

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 10

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

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Glu Tyr Thr Glu Gln Ala	Glu Ala Glu His Ala Glu	Ala Ala Gln Arg
130	135	140
Arg Ala Leu Pro Asn His	Leu His Phe His Ser Leu Glu His	Glu Glu
145	150	155 160
Ala Met Asn Ala Ala Tyr Ser Gly Tyr Val Tyr Thr His Arg Leu Phe		
165	170	175
His Arg Ala Glu Asp Glu Pro Tyr Ala Glu Pro Tyr Ala Asp Tyr Gly		
180	185	190
Gly Leu Gln Glu His Val Tyr Glu Glu Ile Gly Asp Ala Pro Glu Leu		
195	200	205
Glu Ala Arg Asp Gly Leu Arg Leu Tyr Glu Arg Glu Arg Asp Glu Ala		
210	215	220
Ala Ala Tyr Arg Gln Glu Ala Leu Gly Ala Arg Leu His His Tyr Asp		
225	230	235 240
Glu Arg Ser Asp Gly Glu Ser Asp Ser Pro Glu Lys Glu Ala Glu Phe		
245	250	255
Ala Pro Tyr Pro Arg Met Asp Ser Tyr Glu Gln Glu Glu Asp Ile Asp		
260	265	270
Gln Ile Val Ala Glu Val Lys Gln Ser Met Ser Ser Gln Ser Leu Asp		
275	280	285
Lys Ala Ala Glu Asp Met Pro Glu Ala Glu Gln Asp Leu Glu Arg Ala		
290	295	300
Pro Thr Pro Gly Gly Gly His Pro Asp Ser Pro Gly Leu Pro Ala Pro		
305	310	315 320
Ala Gly Gln Gln Gln Arg Val Val Gly Thr Pro Gly Gly Ser Glu Val		
325	330	335
Gly Gln Arg Tyr Ser Lys Glu Lys Arg Asp Ala Ile Ser Leu Ala Ile		
340	345	350
Lys Asp Ile Lys Glu Ala Ile Glu Glu Val Lys Thr Arg Thr Ile Arg		
355	360	365
Ser Pro Tyr Thr Pro Asp Glu Pro Lys Glu Pro Ile Trp Val Met Arg		
370	375	380
Gln Asp Ile Ser Pro Thr Arg Asp Cys Asp Asp Gln Arg Pro Val Asp		
385	390	395 400
Gly Asp Ser Pro Ser Pro Gly Ser Ser Ser Pro Leu Gly Ala Glu Ser		
405	410	415
Ser Ile Thr Pro Leu His Pro Gly Asp Pro Thr Glu Ala Ser Thr Asn		
420	425	430
Lys Glu Ser Arg Lys Ser Leu Ala Ser Phe Pro Thr Tyr Val Glu Val		
435	440	445
Pro Gly Pro Cys Asp Pro Glu Asp Leu Ile Asp Gly Ile Ile Phe Ala		
450	455	460
Ala Asn Tyr Leu Gly Ser Thr Gln Leu Leu Ser Asp Lys Thr Pro Ser		
465	470	475 480
Lys Asn Val Arg Met Met Gln Ala Gln Glu Ala Val Ser Arg Ile Lys		
485	490	495
Thr Ala Gln Lys Leu Ala Lys Ser Arg Lys Lys Ala Pro Glu Gly Glu		
500	505	510
Ser Gln Pro Met Thr Glu Val Asp Leu Phe Ile Ser Thr Gln Arg Ile		
515	520	525

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Lys Val Leu Asn Ala Asp Thr Gln Glu Pro Met Met Asp His Pro Leu
 530 535 540
 Arg Thr Ile Ser Tyr Ile Ala Asp Ile Gly Asn Ile Val Val Leu Met
 545 550 555 560
 Ala Arg Arg Arg Met Pro Arg Ser Asn Ser Gln Glu Asn Val Glu Ala
 565 570 575
 Ser His Pro Ser Gln Asp Ala Lys Arg Gln Tyr Lys Met Ile Cys His
 580 585 590
 Val Phe Glu Ser Glu Asp Ala Gln Leu Ile Ala Gln Ser Ile Gly Gln
 595 600 605
 Ala Phe Ser Val Ala Tyr Gln Glu Phe Leu Arg Ala Asn Gly Ile Asn
 610 615 620
 Pro Glu Asp Leu Ser Gln Lys Glu Tyr Ser Asp Leu Leu Asn Thr Gln
 625 630 635 640
 Asp Met Tyr Asn Asp Asp Leu Ile His Phe Ser Lys Ser Glu Asn Cys
 645 650 655
 Lys Asp Val Phe Ile Glu Lys Gln Lys Gly Glu Ile Leu Ala Ala Val
 660 665 670
 Ile Val Glu Ser Gly Trp Gly Ser Ile Leu Pro Thr Val Ile Ile Ala
 675 680 685
 Asn Met Met His Gly Gly Pro Ala Glu Lys Ser Gly Lys Leu Asn Ile
 690 695 700
 Gly Asp Gln Ile Met Ser Ile Asn Gly Thr Ser Leu Val Gly Leu Pro
 705 710 715 720
 Leu Ser Thr Cys Gln Ser Ile Ile Lys Gly Leu Lys Asn Gln Ser Arg
 725 730 735
 Val Lys Leu Asn Ile Val Arg Cys Pro Pro Val Thr Thr Val Leu Ile
 740 745 750
 Arg Arg Pro Asp Leu Arg Tyr Gln Leu Gly Phe Ser Val Gln Asn Gly
 755 760 765
 Ile Ile Cys Ser Leu Met Arg Gly Gly Ile Ala Glu Arg Gly Gly Val
 770 775 780
 Arg Val Gly His Arg Ile Ile Glu Ile Asn Gly Gln Ser Val Val Ala
 785 790 795 800
 Thr Pro His Glu Lys Ile Val His Ile Leu Ser Asn Ala Val Gly Glu
 805 810 815
 Ile His Met Lys Thr Met Pro Ala Ala Met Tyr Arg Leu Leu Thr Ala
 820 825 830
 Gln Glu Gln Pro Val Tyr Ile
 835

<210> SEQ ID NO 11

<211> LENGTH: 659

<212> TYPE: PRT

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 11

Met Asn His Leu Glu Gly Ser Ala Glu Val Glu Val Ala Asp Glu Ala
 1 5 10 15
 Pro Gly Gly Glu Val Asn Glu Ser Val Glu Ala Asp Leu Glu His Pro
 20 25 30
 Glu Val Glu Glu Glu Gln Gln Pro Ser Pro Pro Pro Ala Gly His
 35 40 45

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Ala	Pro	Glu	Asp	His	Arg	Ala	His	Pro	Ala	Pro	Pro	Pro	Pro	Pro	
50						55				60					
Pro	Gln	Glu	Glu	Glu	Glu	Arg	Gly	Glu	Cys	Leu	Ala	Arg	Ser	Ala	
65					70				75					80	
Ser	Thr	Glu	Ser	Gly	Phe	His	Asn	His	Thr	Asp	Thr	Ala	Glu	Gly	Asp
				85					90					95	
Val	Leu	Ala	Ala	Ala	Arg	Asp	Gly	Tyr	Glu	Ala	Glu	Arg	Ala	Gln	Asp
				100				105						110	
Ala	Asp	Asp	Glu	Ser	Ala	Tyr	Ala	Val	Gln	Tyr	Arg	Pro	Glu	Ala	Glu
			115				120					125			
Glu	Tyr	Thr	Glu	Gln	Ala	Glu	Ala	Glu	His	Ala	Glu	Ala	Ala	Gln	Arg
			130			135					140				
Arg	Ala	Leu	Pro	Asn	His	Leu	His	Phe	His	Ser	Leu	Glu	His	Glu	Glu
				150						155					160
Ala	Met	Asn	Ala	Ala	Tyr	Ser	Gly	Tyr	Val	Tyr	Thr	His	Arg	Leu	Phe
				165					170					175	
His	Arg	Ala	Glu	Asp	Glu	Pro	Tyr	Ala	Glu	Pro	Tyr	Ala	Asp	Tyr	Gly
				180				185					190		
Gly	Leu	Gln	Glu	His	Val	Tyr	Glu	Glu	Ile	Gly	Asp	Ala	Pro	Glu	Leu
			195				200					205			
Glu	Ala	Arg	Asp	Gly	Leu	Arg	Leu	Tyr	Glu	Arg	Glu	Arg	Asp	Glu	Ala
			210			215					220				
Ala	Ala	Tyr	Arg	Gln	Glu	Ala	Leu	Gly	Ala	Arg	Leu	His	His	Tyr	Asp
				230					235						240
Glu	Arg	Ser	Asp	Gly	Glu	Ser	Asp	Ser	Pro	Glu	Lys	Glu	Ala	Glu	Phe
				245					250					255	
Ala	Pro	Tyr	Pro	Arg	Met	Asp	Ser	Tyr	Glu	Gln	Glu	Glu	Asp	Ile	Asp
				260				265					270		
Gln	Ile	Val	Ala	Glu	Val	Lys	Gln	Ser	Met	Ser	Ser	Gln	Ser	Leu	Asp
				275			280					285			
Lys	Ala	Ala	Glu	Asp	Met	Pro	Glu	Ala	Glu	Gln	Asp	Leu	Glu	Arg	Ala
				290			295				300				
Pro	Thr	Pro	Gly	Gly	Gly	His	Pro	Asp	Ser	Pro	Gly	Leu	Pro	Ala	Pro
					310					315					320
Ala	Gly	Gln	Gln	Gln	Arg	Val	Val	Gly	Thr	Pro	Gly	Gly	Ser	Glu	Val
				325					330					335	
Gly	Gln	Arg	Tyr	Ser	Lys	Glu	Lys	Arg	Asp	Ala	Ile	Ser	Leu	Ala	Ile
				340				345					350		
Lys	Asp	Ile	Lys	Glu	Ala	Ile	Glu	Glu	Val	Lys	Thr	Arg	Thr	Ile	Arg
				355			360					365			
Ser	Pro	Tyr	Thr	Pro	Asp	Glu	Pro	Lys	Glu	Pro	Ile	Trp	Val	Met	Arg
						375					380				
Gln	Asp	Ile	Ser	Pro	Thr	Arg	Asp	Cys	Asp	Asp	Gln	Arg	Pro	Val	Asp
					390					395					400
Gly	Asp	Ser	Pro	Ser	Pro	Gly	Ser	Ser	Ser	Pro	Leu	Gly	Ala	Glu	Ser
				405					410					415	
Ser	Ile	Thr	Pro	Leu	His	Pro	Gly	Asp	Pro	Thr	Glu	Ala	Ser	Thr	Asn
				420				425					430		
Lys	Glu	Ser	Arg	Lys	Ser	Leu	Ala	Ser	Phe	Pro	Thr	Tyr	Val	Glu	Val
				435			440						445		

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Pro Gly Pro Cys Asp Pro Glu Asp Leu Ile Asp Gly Ile Ile Phe Ala
 450 455 460
 Ala Asn Tyr Leu Gly Ser Thr Gln Leu Leu Ser Asp Lys Thr Pro Ser
 465 470 475 480
 Lys Asn Val Arg Met Met Gln Ala Gln Glu Ala Val Ser Arg Ile Lys
 485 490 495
 Thr Ala Gln Lys Leu Ala Lys Ser Arg Lys Lys Ala Pro Glu Gly Glu
 500 505 510
 Ser Gln Pro Met Thr Glu Val Asp Leu Phe Ile Ser Thr Gln Arg Ile
 515 520 525
 Lys Val Leu Asn Ala Asp Thr Gln Glu Pro Met Met Asp His Pro Leu
 530 535 540
 Arg Thr Ile Ser Tyr Ile Ala Asp Ile Gly Asn Ile Val Val Leu Met
 545 550 555 560
 Ala Arg Arg Arg Met Pro Arg Ser Asn Ser Gln Glu Asn Val Glu Ala
 565 570 575
 Ser His Pro Ser Gln Asp Ala Lys Arg Gln Tyr Lys Met Ile Cys His
 580 585 590
 Val Phe Glu Ser Glu Asp Ala Gln Leu Ile Ala Gln Ser Ile Gly Gln
 595 600 605
 Ala Phe Ser Val Ala Tyr Gln Glu Phe Leu Arg Ala Asn Gly Ile Asn
 610 615 620
 Pro Glu Asp Leu Ser Gln Lys Glu Tyr Ser Asp Leu Leu Asn Thr Gln
 625 630 635 640
 Asp Met Tyr Asn Asp Asp Leu Ile His Phe Ser Lys Ser Glu Asn Cys
 645 650 655
 Lys Asp Val

<210> SEQ ID NO 12
 <211> LENGTH: 407
 <212> TYPE: PRT
 <213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 12

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

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Ser	His	Pro	Ser	Gln	Asp	Ala	Lys	Arg	Gln	Tyr	Lys	Met	Ile	Cys	His
145					150					155					160
Val	Phe	Glu	Ser	Glu	Asp	Ala	Gln	Leu	Ile	Ala	Gln	Ser	Ile	Gly	Gln
				165					170					175	
Ala	Phe	Ser	Val	Ala	Tyr	Gln	Glu	Phe	Leu	Arg	Ala	Asn	Gly	Ile	Asn
			180					185					190		
Pro	Glu	Asp	Leu	Ser	Gln	Lys	Glu	Tyr	Ser	Asp	Leu	Leu	Asn	Thr	Gln
		195					200					205			
Asp	Met	Tyr	Asn	Asp	Asp	Leu	Ile	His	Phe	Ser	Lys	Ser	Glu	Asn	Cys
	210					215					220				
Lys	Asp	Val	Phe	Ile	Glu	Lys	Gln	Lys	Gly	Glu	Ile	Leu	Gly	Val	Val
225					230					235					240
Ile	Val	Glu	Ser	Gly	Trp	Gly	Ser	Ile	Leu	Pro	Thr	Val	Ile	Ile	Ala
				245					250					255	
Asn	Met	Met	His	Gly	Gly	Pro	Ala	Glu	Lys	Ser	Gly	Lys	Leu	Asn	Ile
			260					265						270	
Gly	Asp	Gln	Ile	Met	Ser	Ile	Asn	Gly	Thr	Ser	Leu	Val	Gly	Leu	Pro
	275						280					285			
Leu	Ser	Thr	Cys	Gln	Ser	Ile	Ile	Lys	Gly	Leu	Lys	Asn	Gln	Ser	Arg
	290						295				300				
Val	Lys	Leu	Asn	Ile	Val	Arg	Cys	Pro	Pro	Val	Thr	Thr	Val	Leu	Ile
305					310					315					320
Arg	Arg	Pro	Asp	Leu	Arg	Tyr	Gln	Leu	Gly	Phe	Ser	Val	Gln	Asn	Gly
			325						330					335	
Ile	Ile	Cys	Ser	Leu	Met	Arg	Gly	Gly	Ile	Ala	Glu	Arg	Gly	Gly	Val
			340					345					350		
Arg	Val	Gly	His	Arg	Ile	Ile	Glu	Ile	Asn	Gly	Gln	Ser	Val	Val	Ala
		355					360					365			
Thr	Pro	His	Glu	Lys	Ile	Val	His	Ile	Leu	Ser	Asn	Ala	Val	Gly	Glu
	370					375					380				
Ile	His	Met	Lys	Thr	Met	Pro	Ala	Ala	Met	Tyr	Arg	Leu	Leu	Thr	Ala
385					390					395					400
Gln	Glu	Gln	Pro	Val	Tyr	Ile									
				405											

<210> SEQ ID NO 13

<211> LENGTH: 750

<212> TYPE: PRT

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 13

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

-continued

Val Thr Tyr	Tyr Ile Arg Tyr Cys	Pro Glu Asp Asp Ser	Tyr Leu Glu
	100	105	110
Gly Met Asp	Cys Asn Gly Glu Glu Tyr	Leu Ala His Gly	Ala His Pro
	115	120	125
Val Asp Thr	Asp Glu Cys Gln Glu Ala	Val Glu Asp Trp	Thr Asp Ser
	130	135	140
Val Gly Pro	His Thr His Ser His Gly	Ala Glu Asn Ser	Gln Glu Tyr
	145	150	155
Pro Asp Ser	His Leu Pro Ile Pro Glu	Asp Asp Pro Thr	Val Leu Glu
	165	170	175
Val His Asp	Gln Glu Glu Asp Gly	His Tyr Cys Pro	Ser Lys Glu Ser
	180	185	190
Tyr Gln Asp	Tyr Tyr Pro Pro Glu	Thr Asn Gly Asn	Thr Gly Gly Ala
	195	200	205
Ser Pro Tyr	Arg Met Arg Arg Gly	Asp Gly Asp Leu	Glu Glu Gln Glu
	210	215	220
Glu Asp Ile	Asp Gln Ile Val Ala Glu	Ile Lys Met Ser	Leu Ser Met
	225	230	235
Thr Ser Ile	Thr Ser Ala Ser Glu	Ala Ser Pro Glu	His Met Pro Glu
	245	250	255
Leu Asp Pro	Gly Asp Ser Thr Glu	Ala Cys Ser Pro	Ser Asp Thr Gly
	260	265	270
Arg Gly Pro	Ser Arg Gln Glu Ala	Arg Pro Lys Ser	Leu Asn Leu Pro
	275	280	285
Pro Glu Val	Lys His Ser Gly	Asp Pro Gln Arg	Gly Leu Lys Thr Lys
	290	295	300
Thr Arg Thr	Pro Glu Glu Arg Pro	Lys Trp Pro Gln	Glu Gln Val Cys
	305	310	315
Asn Gly Leu	Glu Gln Pro Arg Lys	Gln Gln Arg Ser	Asp Leu Asn Gly
	325	330	335
Pro Thr Asp	Asn Asn Ile Pro Glu	Thr Lys Lys Val	Ala Ser Phe
	340	345	350
Pro Ser Phe	Val Ala Val Pro Gly	Pro Cys Glu Pro	Glu Asp Leu Ile
	355	360	365
Asp Gly Ile	Ile Phe Ala Ala Asn	Tyr Leu Gly Ser	Thr Gln Leu Leu
	370	375	380
Ser Glu Arg	Asn Pro Ser Lys	Asn Ile Arg Met	Met Gln Ala Gln Glu
	385	390	395
Ala Val Ser	Arg Val Lys Arg Met	Gln Lys Ala Ala	Lys Ile Lys Lys
	405	410	415
Lys Ala Asn	Ser Glu Gly Asp	Ala Gln Thr Leu	Thr Glu Val Asp Leu
	420	425	430
Phe Ile Ser	Thr Gln Arg Ile Lys	Val Leu Asn Ala	Asp Thr Gln Glu
	435	440	445
Thr Met Met	Asp His Ala Leu Arg	Thr Ile Ser Tyr	Ile Ala Asp Ile
	450	455	460
Gly Asn Ile	Val Val Leu Met	Ala Arg Arg Arg	Met Pro Arg Ser Ala
	465	470	475
Ser Gln Asp	Cys Ile Glu Thr Thr	Pro Gly Ala Gln	Glu Gly Lys Lys
	485	490	495

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<210> SEQ ID NO 14
<211> LENGTH: 750
<212> TYPE: PRT
<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 14

Met Ala His Arg Lys Arg Gln Ser Thr Ala Ser Ser Met Leu Asp His
 1             5             10             15

Arg Ala Arg Pro Gly Pro Ile Pro His Asp Gln Glu Pro Glu Asn Glu
 20             25             30

Asp Thr Glu Leu Pro Leu Glu Ser Tyr Val Pro Thr Gly Leu Glu Leu
 35             40             45

Gly Thr Leu Arg Pro Asp Ser Ser Pro Thr Pro Glu Glu Gln Glu Cys His
 50             55             60

Asn His Ser Pro Asp Gly Asp Ser Ser Ser Asp Tyr Val Asn Asn Thr
 65             70             75             80

Ser Glu Glu Glu Asp Tyr Asp Glu Gly Leu Pro Glu Glu Glu Glu Gly
 85             90             95

Val Thr Tyr Tyr Ile Arg Tyr Cys Pro Glu Asp Asp Ser Tyr Leu Glu
 100            105            110

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Gly	Met	Asp	Cys	Asn	Gly	Glu	Glu	Tyr	Leu	Ala	His	Gly	Ala	His	Pro
	115						120					125			
Val	Asp	Thr	Asp	Glu	Cys	Gln	Glu	Ala	Val	Glu	Asp	Trp	Thr	Asp	Ser
	130					135					140				
Val	Gly	Pro	His	Thr	His	Ser	His	Gly	Ala	Glu	Asn	Ser	Gln	Glu	Tyr
	145				150					155					160
Pro	Asp	Ser	His	Leu	Pro	Ile	Pro	Glu	Asp	Pro	Thr	Val	Leu	Glu	
				165					170				175		
Val	His	Asp	Gln	Glu	Glu	Asp	Gly	His	Tyr	Cys	Pro	Ser	Lys	Glu	Ser
			180					185					190		
Tyr	Gln	Asp	Tyr	Tyr	Pro	Pro	Glu	Thr	Asn	Gly	Asn	Thr	Gly	Gly	Ala
	195						200					205			
Ser	Pro	Tyr	Arg	Met	Arg	Arg	Gly	Asp	Gly	Asp	Leu	Glu	Glu	Gln	Glu
	210					215					220				
Glu	Asp	Ile	Asp	Gln	Ile	Val	Ala	Glu	Ile	Lys	Met	Ser	Leu	Ser	Met
	225				230					235					240
Thr	Ser	Ile	Thr	Ser	Ala	Ser	Glu	Ala	Ser	Pro	Glu	His	Met	Pro	Glu
				245					250					255	
Leu	Asp	Pro	Gly	Asp	Ser	Thr	Glu	Ala	Cys	Ser	Pro	Ser	Asp	Thr	Gly
			260					265					270		
Arg	Gly	Pro	Ser	Arg	Gln	Glu	Ala	Arg	Pro	Lys	Ser	Leu	Asn	Leu	Pro
	275						280					285			
Pro	Glu	Val	Lys	His	Ser	Gly	Asp	Pro	Gln	Arg	Gly	Leu	Lys	Thr	Lys
	290					295					300				
Thr	Arg	Thr	Pro	Glu	Glu	Arg	Pro	Lys	Trp	Pro	Gln	Glu	Gln	Val	Cys
	305				310					315					320
Asn	Gly	Leu	Glu	Gln	Pro	Arg	Lys	Gln	Gln	Arg	Ser	Asp	Leu	Asn	Gly
				325					330					335	
Pro	Thr	Asp	Asn	Asn	Asn	Ile	Pro	Glu	Thr	Lys	Lys	Val	Ala	Ser	Phe
			340					345					350		
Pro	Ser	Phe	Val	Ala	Val	Pro	Gly	Pro	Cys	Glu	Pro	Glu	Asp	Leu	Ile
		355					360					365			
Asp	Gly	Ile	Ile	Phe	Ala	Ala	Asn	Tyr	Leu	Gly	Ser	Thr	Gln	Leu	Leu
	370					375					380				
Ser	Glu	Arg	Asn	Pro	Ser	Lys	Asn	Ile	Arg	Met	Met	Gln	Ala	Gln	Glu
	385				390					395					400
Ala	Val	Ser	Arg	Val	Lys	Arg	Met	Gln	Lys	Ala	Ala	Lys	Ile	Lys	Lys
				405					410					415	
Lys	Ala	Asn	Ser	Glu	Gly	Asp	Ala	Gln	Thr	Leu	Thr	Glu	Val	Asp	Leu
			420					425					430		
Phe	Ile	Ser	Thr	Gln	Arg	Ile	Lys	Val	Leu	Asn	Ala	Asp	Thr	Gln	Glu
	435						440					445			
Thr	Met	Met	Asp	His	Ala	Leu	Arg	Thr	Ile	Ser	Tyr	Ile	Ala	Asp	Ile
	450					455					460				
Gly	Asn	Ile	Val	Val	Leu	Met	Ala	Arg	Arg	Arg	Met	Pro	Arg	Ser	Ala
	465				470					475					480
Ser	Gln	Asp	Cys	Ile	Glu	Thr	Thr	Pro	Gly	Ala	Gln	Glu	Gly	Lys	Lys
				485					490					495	
Gln	Tyr	Lys	Met	Ile	Cys	His	Val	Phe	Glu	Ser	Glu	Asp	Ala	Gln	Leu
			500					505						510	

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Ile	Ala	Gln	Ser	Ile	Gly	Gln	Ala	Phe	Ser	Val	Ala	Tyr	Gln	Glu	Phe
	515						520					525			
Leu	Arg	Ala	Asn	Gly	Ile	Asn	Pro	Glu	Asp	Leu	Ser	Gln	Lys	Glu	Tyr
	530						535				540				
Ser	Asp	Ile	Ile	Asn	Thr	Gln	Glu	Met	Tyr	Asn	Asp	Asp	Leu	Ile	His
545					550					555					560
Phe	Ser	Asn	Ser	Glu	Asn	Cys	Lys	Glu	Leu	Gln	Leu	Glu	Lys	His	Lys
				565					570					575	
Gly	Glu	Ile	Leu	Ala	Ala	Val	Val	Val	Glu	Ser	Gly	Trp	Gly	Ser	Ile
			580					585					590		
Leu	Pro	Thr	Val	Ile	Leu	Ala	Asn	Met	Met	Asn	Gly	Gly	Pro	Ala	Ala
		595					600					605			
Arg	Ser	Gly	Lys	Leu	Ser	Ile	Gly	Asp	Gln	Ile	Met	Ser	Ile	Asn	Gly
	610						615				620				
Thr	Ser	Leu	Val	Gly	Leu	Pro	Leu	Ala	Thr	Cys	Gln	Gly	Ile	Ile	Lys
625					630					635					640
Gly	Leu	Lys	Asn	Gln	Thr	Gln	Val	Lys	Leu	Asn	Ile	Val	Ser	Cys	Pro
			645						650					655	
Pro	Val	Thr	Thr	Val	Leu	Ile	Lys	Arg	Pro	Asp	Leu	Lys	Tyr	Gln	Leu
			660					665					670		
Gly	Phe	Ser	Val	Gln	Asn	Gly	Ile	Ile	Cys	Ser	Leu	Met	Arg	Gly	Gly
		675					680					685			
Ile	Ala	Glu	Arg	Gly	Gly	Val	Arg	Val	Gly	His	Arg	Ile	Ile	Glu	Ile
	690						695				700				
Asn	Gly	Gln	Ser	Val	Val	Ala	Thr	Ala	His	Glu	Lys	Ile	Val	Gln	Ala
705					710					715					720
Leu	Ser	Asn	Ser	Val	Gly	Glu	Ile	His	Met	Lys	Thr	Met	Pro	Ala	Ala
				725					730					735	
Met	Phe	Arg	Leu	Leu	Thr	Gly	Gln	Glu	Thr	Pro	Leu	Tyr	Ile		
		740						745					750		

<210> SEQ ID NO 15

<211> LENGTH: 570

<212> TYPE: PRT

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 15

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

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Val	Asp	Thr	Asp	Glu	Cys	Gln	Glu	Ala	Val	Glu	Asp	Trp	Thr	Asp	Ser	130	135	140
Val	Gly	Pro	His	Thr	His	Ser	His	Gly	Ala	Glu	Asn	Ser	Gln	Glu	Tyr	145	150	155
Pro	Asp	Ser	His	Leu	Pro	Ile	Pro	Glu	Asp	Pro	Thr	Val	Leu	Glu		165	170	175
Val	His	Asp	Gln	Glu	Glu	Asp	Gly	His	Tyr	Cys	Pro	Ser	Lys	Glu	Ser	180	185	190
Tyr	Gln	Asp	Tyr	Tyr	Pro	Pro	Glu	Thr	Asn	Gly	Asn	Thr	Gly	Gly	Ala	195	200	205
Ser	Pro	Tyr	Arg	Met	Arg	Arg	Gly	Asp	Gly	Asp	Leu	Glu	Glu	Gln	Glu	210	215	220
Glu	Asp	Ile	Asp	Gln	Ile	Val	Ala	Glu	Ile	Lys	Met	Ser	Leu	Ser	Met	225	230	235
Thr	Ser	Ile	Thr	Ser	Ala	Ser	Glu	Ala	Ser	Pro	Glu	His	Met	Pro	Glu	245	250	255
Leu	Asp	Pro	Gly	Asp	Ser	Thr	Glu	Ala	Cys	Ser	Pro	Ser	Asp	Thr	Gly	260	265	270
Arg	Gly	Pro	Ser	Arg	Gln	Glu	Ala	Arg	Pro	Lys	Ser	Leu	Asn	Leu	Pro	275	280	285
Pro	Glu	Val	Lys	His	Ser	Gly	Asp	Pro	Gln	Arg	Gly	Leu	Lys	Thr	Lys	290	295	300
Thr	Arg	Thr	Pro	Glu	Glu	Arg	Pro	Lys	Trp	Pro	Gln	Glu	Gln	Val	Cys	305	310	315
Asn	Gly	Leu	Glu	Gln	Pro	Arg	Lys	Gln	Gln	Arg	Ser	Asp	Leu	Asn	Gly	325	330	335
Pro	Thr	Asp	Asn	Asn	Asn	Ile	Pro	Glu	Thr	Lys	Lys	Val	Ala	Ser	Phe	340	345	350
Pro	Ser	Phe	Val	Ala	Val	Pro	Gly	Pro	Cys	Glu	Pro	Glu	Asp	Leu	Ile	355	360	365
Asp	Gly	Ile	Ile	Phe	Ala	Ala	Asn	Tyr	Leu	Gly	Ser	Thr	Gln	Leu	Leu	370	375	380
Ser	Glu	Arg	Asn	Pro	Ser	Lys	Asn	Ile	Arg	Met	Met	Gln	Ala	Gln	Glu	385	390	395
Ala	Val	Ser	Arg	Val	Lys	Arg	Met	Gln	Lys	Ala	Ala	Lys	Ile	Lys	Lys	405	410	415
Lys	Ala	Asn	Ser	Glu	Gly	Asp	Ala	Gln	Thr	Leu	Thr	Glu	Val	Asp	Leu	420	425	430
Phe	Ile	Ser	Thr	Gln	Arg	Ile	Lys	Val	Leu	Asn	Ala	Asp	Thr	Gln	Glu	435	440	445
Thr	Met	Met	Asp	His	Ala	Leu	Arg	Thr	Ile	Ser	Tyr	Ile	Ala	Asp	Ile	450	455	460
Gly	Asn	Ile	Val	Val	Leu	Met	Ala	Arg	Arg	Arg	Met	Pro	Arg	Ser	Ala	465	470	475
Ser	Gln	Asp	Cys	Ile	Glu	Thr	Thr	Pro	Gly	Ala	Gln	Glu	Gly	Lys	Lys	485	490	495
Gln	Tyr	Lys	Met	Ile	Cys	His	Val	Phe	Glu	Ser	Glu	Asp	Ala	Gln	Leu	500	505	510
Ile	Ala	Gln	Ser	Ile	Gly	Gln	Ala	Phe	Ser	Val	Ala	Tyr	Gln	Glu	Phe	515	520	525

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Leu Arg Ala Asn Gly Ile Asn Pro Glu Asp Leu Ser Gln Lys Glu Tyr
530 535 540

Ser Asp Ile Ile Asn Thr Gln Glu Met Tyr Asn Asp Asp Leu Ile His
545 550 555 560

Phe Ser Asn Ser Glu Asn Cys Lys Glu Leu
565 570

<210> SEQ ID NO 16

<211> LENGTH: 408

<212> TYPE: PRT

<213> ORGANISM: rattus norvegicus

<400> SEQUENCE: 16

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ile Gln Ile
1 5 10 15

Trp Tyr Pro Cys Glu Pro Glu Asp Leu Ile Asp Gly Ile Ile Phe Ala
20 25 30

Ala Asn Tyr Leu Gly Ser Thr Gln Leu Leu Ser Glu Arg Asn Pro Ser
35 40 45

Lys Asn Ile Arg Met Met Gln Ala Gln Glu Ala Val Ser Arg Val Lys
50 55 60

Arg Met Gln Lys Ala Ala Lys Ile Lys Lys Lys Ala Asn Ser Glu Gly
65 70 75 80

Asp Ala Gln Thr Leu Thr Glu Val Asp Leu Phe Ile Ser Thr Gln Arg
85 90 95

Ile Lys Val Leu Asn Ala Asp Thr Gln Glu Thr Met Met Asp His Ala
100 105 110

Leu Arg Thr Ile Ser Tyr Ile Ala Asp Ile Gly Asn Ile Val Val Leu
115 120 125

Met Ala Arg Arg Arg Met Pro Arg Ser Ala Ser Gln Asp Cys Ile Glu
130 135 140

Thr Thr Pro Gly Ala Gln Glu Gly Lys Lys Gln Tyr Lys Met Ile Cys
145 150 155 160

His Val Phe Glu Ser Glu Asp Ala Gln Leu Ile Ala Gln Ser Ile Gly
165 170 175

Gln Ala Phe Ser Val Ala Tyr Gln Glu Phe Leu Arg Ala Asn Gly Ile
180 185 190

Asn Pro Glu Asp Leu Ser Gln Lys Glu Tyr Ser Asp Ile Ile Asn Thr
195 200 205

Gln Glu Met Tyr Asn Asp Asp Leu Ile His Phe Ser Asn Ser Glu Asn
210 215 220

Cys Lys Glu Leu Gln Leu Glu Lys His Lys Gly Glu Ile Leu Gly Val
225 230 235 240

Val Val Val Glu Ser Gly Trp Gly Ser Ile Leu Pro Thr Val Ile Leu
245 250 255

Ala Asn Met Met Asn Gly Gly Pro Ala Ala Arg Ser Gly Lys Leu Ser
260 265 270

Ile Gly Asp Gln Ile Met Ser Ile Asn Gly Thr Ser Leu Val Gly Leu
275 280 285

Pro Leu Ala Thr Cys Gln Gly Ile Ile Lys Gly Leu Lys Asn Gln Thr
290 295 300

Gln Val Lys Leu Asn Ile Val Ser Cys Pro Pro Val Thr Thr Val Leu
305 310 315 320

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Ile Lys Arg Pro Asp Leu Lys Tyr Gln Leu Gly Phe Ser Val Gln Asn
325 330 335
Gly Ile Ile Cys Ser Leu Met Arg Gly Gly Ile Ala Glu Arg Gly Gly
340 345 350
Val Arg Val Gly His Arg Ile Ile Glu Ile Asn Gly Gln Ser Val Val
355 360 365
Ala Thr Ala His Glu Lys Ile Val Gln Ala Leu Ser Asn Ser Val Gly
370 375 380
Glu Ile His Met Lys Thr Met Pro Ala Ala Met Phe Arg Leu Leu Thr
385 390 395 400
Gly Gln Glu Thr Pro Leu Tyr Ile
405

<210> SEQ ID NO 17
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: homo sapiens
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (0)...(0)
<223> OTHER INFORMATION: Binds to PTB binding domain

<400> SEQUENCE: 17

Asn Pro Thr Tyr
1

<210> SEQ ID NO 18
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 18

Cys Met Asp Gln Leu Ala Phe His Gln Phe Tyr Ile
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 19

Cys Met Asp Thr Leu Ala Ser His Gln Leu Tyr Ile
1 5 10

<210> SEQ ID NO 20
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: drosophila melanogaster

<400> SEQUENCE: 20

Cys Met Glu Asp Leu Ser Ala Lys Gln Val Phe Ile
1 5 10

<210> SEQ ID NO 21
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 21

Cys Gly Ser Leu Ile Ser Arg Arg Ala Val Tyr Val

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1	5	10
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<210> SEQ ID NO 22
 <211> LENGTH: 12
 <212> TYPE: PRT
 <213> ORGANISM: homo sapiens

 <400> SEQUENCE: 22

 Cys Trp Phe Ser Ile Thr Asn Trp Leu Trp Tyr Ile
 1 5 10

<210> SEQ ID NO 23
 <211> LENGTH: 14
 <212> TYPE: PRT
 <213> ORGANISM: homo sapiens

 <400> SEQUENCE: 23

 Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln
 1 5 10

<210> SEQ ID NO 24
 <211> LENGTH: 14
 <212> TYPE: PRT
 <213> ORGANISM: homo sapiens

 <400> SEQUENCE: 24

 Gln Asn Gly Tyr Glu Asn Ala Thr Ala Lys Phe Phe Glu Gln
 1 5 10

We claim:

1. A method of identifying an agent that affects the cleavage of amyloid- β precursor protein (APP) comprising providing a cell containing APP modified in the C-terminal cytoplasmic tail to allow detection of nuclear localization of said cytoplasmic tail; contacting said cell with a candidate agent; and measuring nuclear localization of said C-terminal cytoplasmic tail, wherein an increase or decrease in nuclear localization in the presence of said candidate agent relative to nuclear localization in the absence of said candidate agent is indicative of an agent that affects the cleavage of APP.

2. The method of claim 1 wherein APP is modified to comprise a DNA-binding domain of a transcription factor and a transcriptional activator of the same or a different transcription factor, and wherein said cell further contains an indicator gene operably linked to a nucleic acid comprising a binding site for said DNA-binding domain.

3. The method of claim 2 wherein said DNA-binding domain is Gal4 or LexA.

4. The method of claim 2 wherein said transcriptional activator is VP16.

5. The method of claim 1 wherein said APP is modified to comprise a DNA-binding domain of a transcription factor and wherein said cell further comprises an indicator gene operably linked to a nucleic acid comprising a binding site for said DNA-binding domain, and wherein Fe65 is provided to said cell.

6. The method of claim 5 wherein said DNA-binding domain is Gal4 or LexA.

7. The method of claim 2 wherein nuclear localization is measured by measuring expression of said indicator gene.

8. The method of claim 5 wherein nuclear localization is measured by measuring expression of said indicator gene.

9. The method of claim 1 wherein said cell is a eukaryotic cell.

10. The method of claim 1 wherein said cell is a mammalian cell.

11. The method of claim 1 wherein said cell is a human cell.

12. A method of identifying an agent that affects the cleavage of amyloid- β precursor protein (APP) comprising co-transfecting a cell with (a) a nucleic acid encoding a modified APP, wherein said modified APP comprises Gal4 and VP16 in the C-terminal cytoplasmic tail, and (b) a nucleic acid encoding an indicator gene under the control of one or more copies of a Gal4 regulatory element; contacting said cell with a candidate agent; and measuring expression of said indicator gene, wherein an increase or decrease in expression in the presence of said candidate agent relative to the absence of said agent is indicative of an agent that affects cleavage of APP.

13. A method of identifying an agent that affects the cleavage of amyloid- β precursor protein (APP) comprising providing a cell containing APP and Tip60 modified to allow detection of nuclear localization of a C-terminal cytoplasmic cleavage product of APP; contacting said cell with a candidate agent; and measuring nuclear localization of said C-terminal cytoplasmic cleavage product, wherein an increase or decrease in nuclear localization in the presence of said agent relative to nuclear localization in the absence of said agent is indicative of an agent that affects the cleavage of APP.

14. The method of claim 13 wherein said Tip60 is modified by fusion with the DNA binding domain of a transcriptional activator, and wherein said cell further contains Fe65.

15. The method of claim 14 wherein said DNA binding domain is Gal4 or LexA.

16. The method of claim 14 wherein said cell further contains an indicator gene operably linked to a nucleic acid comprising a binding site for said DNA binding domain.

17. The method of claim 16 wherein said nuclear localization is measured by measuring expression of said indicator gene.

18. The method of claim 13 wherein said cell is a eukaryotic cell.

19. The method of claim 13 wherein said cell is a mammalian cell.

20. The method of claim 13 wherein said cell is a human cell.

21. A method of identifying an agent that affects the cleavage of amyloid- β precursor protein (APP) comprising co-transfecting a cell with (a) a nucleic acid encoding APP, (b) a nucleic acid encoding Fe65, (c) a nucleic acid encoding a fusion protein of Tip60 and Gal4, and (d) a nucleic acid encoding an indicator gene under the control of one or more copies of a Gal4 regulatory element; contacting said cell with a candidate agent; and measuring expression of said indicator gene, wherein an increase or decrease in expression in the presence of said candidate agent relative to the absence of said agent is indicative of an agent that affects the cleavage of APP.

22. A vector comprising a first nucleic acid encoding amyloid- β precursor protein (APP) operably linked to a promoter, wherein a second nucleic acid encoding a heterologous DNA-binding domain of a transcription factor is contained within the region of the first nucleic acid that encodes the C-terminal cytoplasmic tail of APP.

23. A vector comprising a nucleic acid encoding amyloid- β precursor protein (APP) operably linked to a promoter wherein a nucleic acid module encoding a heterologous DNA-binding domain of a transcription factor and a transcriptional activator of the same or a different transcription factor is contained within the region of the nucleic acid that encodes the C-terminal cytoplasmic tail of APP.

24. The vector of claim 28 wherein the DNA-binding domain is Gal4 or LexA.

25. The vector of claim 23 wherein the DNA-binding domain is Gal4 or LexA and the transcriptional activator is VP16.

26. A vector comprising a nucleic acid encoding Tip60 and a heterologous DNA binding domain of a transcription factor.

27. The vector of claim 26 wherein said DNA binding domain is Gal4.

28. A cell comprising the vector of claim 22.

29. A cell comprising the vector of claim 23.

30. A cell comprising the vector of claim 24.

31. An agent that affects the cleavage of amyloid- β precursor protein (APP) identified by a method comprising providing a cell containing APP modified in the C-terminal cytoplasmic tail to allow detection of nuclear localization of said cytoplasmic tail; contacting said cell with a candidate agent; and measuring nuclear localization of said C-terminal cytoplasmic tail, wherein an increase or decrease in nuclear localization in the presence of said candidate agent relative to nuclear localization in the absence of said candidate agent is indicative of an agent that affects the cleavage of APP.

32. An agent that affects the cleavage of amyloid- β precursor protein (APP) identified by a method comprising providing a cell containing APP and a protein that interacts with APP to activate transcription, wherein the protein is modified to allow detection of nuclear localization of a C-terminal cytoplasmic cleavage product of APP; contacting said cell with a candidate agent; and measuring nuclear localization of said C-terminal cytoplasmic cleavage product, wherein an increase or decrease in nuclear localization in the presence of said agent relative to nuclear localization in the absence of said agent is indicative of an agent that affects the cleavage of APP.

33. A composition comprising the agent of claim 31.

34. A composition comprising the agent of claim 32.

35. A kit comprising a first compartment containing cells comprising a vector wherein said vector comprises a nucleic acid encoding amyloid- β precursor protein (APP) operably linked to a promoter wherein a nucleic acid module encoding a heterologous DNA-binding domain of a transcription factor and a transcriptional activator of the same or a different transcription factor is contained within the region of the nucleic acid that encodes the C-terminal cytoplasmic tail of APP.

36. The kit of claim 35 wherein said cells further contain a nucleic acid encoding an indicator gene under the control of a regulatory element for said DNA-binding domain.

37. A kit comprising a first compartment containing a vector comprising a nucleic acid encoding amyloid- β precursor protein (APP) operably linked to a promoter wherein a nucleic acid module encoding a heterologous DNA-binding domain of a transcription factor and a transcriptional activator of the same or a different transcription factor is contained within the region of the nucleic acid that encodes the C-terminal cytoplasmic tail of APP.

38. The kit of claim 37 further comprising a second compartment containing a reporter plasmid comprising a nucleic acid encoding an indicator gene under the control of a regulatory element for said DNA-binding domain.

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