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(54) **JNK3 MODULATORS AND METHODS OF USE**

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(75) Inventors: **Roger J. Davis**, Princeton, MA (US);
Alan J. Whitmarsh, Manchester (GB)

Publication Classification

Correspondence Address:

J. Peter Fasse

FISH & RICHARDSON P.C.

Suite 3300

60 South Sixth Street

Minneapolis, MN 55402 (US)

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(57)

ABSTRACT

(73) Assignee: **University of Massachusetts, a Massachusetts corporation**

The c-Jun NH₂-terminal kinase (JNK) group of MAP kinases are activated by exposure of cells to environmental stress. The role of JNK in the brain was examined by targeted disruption of the gene that encodes the neuronal isoform JNK3. It was found that JNK3 is required for the normal response to seizure activity. Methods of screening for molecules and compounds that decrease JNK3 expression or activity are described. Such molecules or compounds are useful for treating disorders involving excitotoxicity such as seizure disorders, Alzheimer's disease, Huntington disease, Parkinson's disease, and ischaemia.

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(22) Filed: **Jul. 12, 2005**

Related U.S. Application Data

(63) Continuation of application No. 09/165,522, filed on Oct. 2, 1998, now Pat. No. 6,943,000.

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(SEQ ID NO: 1)

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gcagttggat gaaagagaac acacaattga agaattggaa gaacttatct acaaggaagt
aatgaattca gaagaaaaga ctaaaaatgg ttagtaaaaa ggacagcctt ctcttcagg
tgcagcagtg aacagcagtg agagtctccc tccatcctcg tctgtcaatg acatctctc
catgtccacc gaccagaccc tggcatctga cactgacagc agcctggaag cctcggcagg
acctctgggt tgttgcaggt gactagccgc ctgcctgcga aaccagcgt tcttcaggag
atgat

(SEQ ID NO: 1)

FIG. 1A

MSLHFLYYCSEPTLDVKIAFCQGFDKQVDVSYIAKHYNMSKSKVDNQFY SVEVGDSTFTVL
KRYQNLKPIGSGAQGIVCAAYDAVLDRNVAIKKLSRPFQNOTHAKRAYRELVL MKCVNHKN
IISLLNVFTPQKTLEEFQDVYLVME LMDANLCQVIQMELDHERMSYLLYQMLCGIKHLHSA
GIIHRDLKPSNIVVKS DCTLKILDFGLARTAGTSFMMPYVVTRY YRAPEVILGMGYKENV
DIWSVGCIMGEMVRHKILFPGRDYIDQWNKVIEQLGTPCPEFMKKLQPTVRNYVENR PKYA
GLTFPKLFPDSLFPADSEHNKLKASQARDLLSKMLVIDPAKRISVDDALQHPYINVWYDPA
EVEAPPPQIYDKQLDEREHTIEEWKELIYKEVMNSEEKTKNGVVKGQPSPSGA AVNSSESL
PPSSSVNDISSMSTDQTLASDTDSSLEASAGPLGCCR
(SEQ ID NO: 2)

FIG. 1B

GAGAAATGGCGTGGCAGGGGACCCAGCGAGCCCAGAGGGATTTTGCCGCTGCTTCCTCTAC
CCCTGTATTTACGCGAGCTCTCTAAATTGACTCAGCTCCAGGCTAGTGTGAGAAACACCAA
CAGCAGGCCCCATCTCAGATCTTCACTATGGCAACTTATGCAAGAACTGTTGAATTAGACC
CGTTTCCTATAGATGAGAAACCATAACAAGCTGTGGTATTTATGAGCCTCCATTTCTTATAC
TACTGCAGTGAACCAACATTGGATGTGAAAATTGCCTTTTGTGAGGGATTTCGATAAACAAAG
TGGATGTGTCATATATTGCCAAACATTACAACATGAGCAAAAGCAAAGTTGACAACCAGTT
CTACAGTGTGGAAGTGGGAGACTCAACCTTCACAGTTCTCAAGCGCTACCAGAATCTAAAG
CCTATTGGCTCTGGGGCTCAGGGCATAGTTTGTGCGCGTATGATGCTGTCCTTGACAGAA
ATGTGGCCATTAAAGAAGCTCAGCAGACCCTTTCAGAACCAACACATGCCAAGAGAGCGTA
CCGGGAGCTGGTCCTCATGAAGTGTGTGAACCATAAAAACATTATTAGTTTATTAAATGTC
TTCACACCCCCAGAAAACGCTGGAGGAGTTCCAAGATGTTTACTTAGTAATGGAAGTGTGG
ATGCCAACTTATGTCAAGTGATTCAGATGGAATTAGACCATGAGCGAATGTCTTACCTGCT
GTACCAAATGTTGTGTGGCATTAAAGCACCTCCATTCTGCTGGAATTATTCACAGGGATTTA
AAACCAAGTAACATTGTAGTCAAGTCTGATTGCACATTGAAAATCCTGGACTTTGGACTGG
CCAGGACAGCAGGCACAAGCTTCATGATGACTCCATATGTGGTGACACGTTATTACAGAGC
CCCTGAGGTCATCCTGGGGATGGGCTACAAGGAGAACGTGGATATATGGTCTGTGGGATGC
ATTATGGGAGAAATGGTTCGCCACAAAATCCTCTTTCAGGAAGGGACTATATTGACCAGT
GGAATAAGGTAATTGAACAACCTAGGAACACCATGTCCAGAATTCATGAAGAAATTGCAACC
CACAGTAAGAACTATGTGGAGAATCGGCCCAAGTATGCGGGGACTCACCTTCCCCAAACTC
TTCCCAGATTCCCTCTTCCCAGCGGACTCCGAGCACATAAACTCAAAGCCAGCCAAGCCA
GGGACTTGTTGTCAAAGATGCTAGTGATTGACCCAGCAAAAAGAATATCAGTGGACGACGC
CTTACAGCATCCCTACATCAACGTCTGGTATGACCCAGCCGAAGTGGAGGCGCCTCCACCT
CAGATATATGACAAGCAGTTGGATGAAAGAGAACACACAATTGAAGAATGGAAAGAACTTA
TCTACAAGGAAGTAATGAATTCAGAAGAAAAGACTAAAAATGGTGTAGTAAAAGGACAGCC
TTCTCCTTCAGGTGCAGCAGTGAACAGCAGTGAGAGTCTCCCTCCATCCTCGTCTGTCAAT
GACATCTCCTCCATGTCCACCGACCAGACCCTGGCATCTGACACTGACAGCAGCCTGGAAG
CCTCGGCAGGACCCCTGGGTTGTTGCAGGTGACTAGCCGCTGCTGCGAAACCCAGCGTT
CTTCAGGAGATGATGTGATGGAACACACACACACGACACACACACACACAAATGCA
GACACACAACATCAAGAAAACAGCAAGGGAGAGAATCCAAGCCTAAAATTAAATAAATCTT
TCAGCCTGCTTCTTCCCCAGGGTTCTGTATTGCAGCTAAGCTCAAATGTATATTTAACTTC
TAGTTGCTCTTGCTTTGGTCTTCTTCCAATGATGCTTACTACAGAAAGCAAATCAGACACA
ATTAGAGAAGCCTTTTCCATAAAGTGTAATTTTAATGGCTGCAAAACCGGCAACCTGTAAC
TGCCCTTTTAAATGGCATGACAAGGTGTGCAGTGGCCCCATCCAGCATGTGTGTGTCTCTA
TCTTGCACTCTACCTGCTCCTTGGCCTAGTCAGATGGATGTAGATACAGATCCGCATGTGTC
TGTATTCATACAGCACTACTTACTTAGAGATGCTACTCTCAGTGTCTCAGGGCTCTACCA
AGACATAATGCACTGGGGTACCACATGGTCCATTTTCATGTGATCTATTACTCTGACATAAA
CCCATCTGTAATATATTGCCAGTATATAAGCTGTTTGTGTTAATTGATTAAACTGTAT
GTCTTATAAGAAAACATGTAAAGGGGGAATATATTGGGGGAGTGAGCTCTCTCAGACCCTT
GAAGATGTAGCTTCCAAATTTGAATGGATTAAATGGCACCTGTATACCA

(SEQ ID NO: 3)

FIG. 1C

atctcagatc ttcactatgg caacttatgc aagaaactgt tgaattagac cegtttccta
tagatgagaa accatacaag ctgtggtatt tatgagcctc catttcttatactactgcag
tgaaccaaca ttggatgtga aaattgcctt ttgtcaggga ttcgataaac aagtggatgt
gtcatatatt gccaaacatt acaacatgag caaaagcaaa gttgacaacc agttctacag
tgtggaagtg ggagactcaa ccttcacagt tctcaagcgc taccagaatc taaagcctat
tggctctggg gctcagggca tagtttgtgc cgcgtatgat gctgtccttg acagaaatgt
ggccattaag aagctcagca gaccctttca gaaccaaaca catgccaaaga gagcgtaccg
ggagctggtc ctcatgaagt gtgtgaacca taaaaacatt attagtttat taaatgtctt
cacaccccag aaaacgctgg aggagttcca agatgtttac ttagtaatgg aactgatgga
tgccaactta tgtcaagtga ttcagatgga attagaccat gagcgaatgt cttacctgct
gtaccaaagt ttgtgtggca ttaagcacct ccattctgct ggaattatc acagggattt
aaaaccaagt aacattgtag tcaagtctga ttgcacattg aaaatcctgg actttggact
ggccaggaca gcaggcacia gcttcatgat gactccatat gtggtgacac gttattacag
agcccctgag gtcacctctg ggatgggcta caaggagaac gtggatatat ggtctgtggg
atgcattatg ggagaaatgg ttgccacaa aatcctcttt ccaggaaggg actatattga
ccagtggaat aaggtaattg aacaactagg aacaccatgt ccagaattca tgaagaaatt
gcaaccacac gtaagaaact atgtggagaa tcggcccaag tatgcgggac tcaccttccc
caaactcttc ccagattccc tcttcccagc ggactccgag cacaataaac tcaaagccag
ccaagccagg gacttgttgt caaagatgct agtgattgac ccagcaaaaa gaatatcagt
ggacgacgcc ttacagcatc cctacatcaa cgtctggtat gacctagccg aagtggaggc
gcctccacct cagatatatg acaagcagtt ggatgaaaga gaacacacaa ttgaagaatg
gaaagaactt atctacaagg aagtaatgaa ttcagaagaa aagactaaaa atgggtgtagt
aaaaggacag ccttctcctt cagcacaggt gcagcagtga acagcagtga gagtctccct
ccatcctcgt ctgtcaatga catctcctcc atgtccaccg accagaccct ggcattctgac
actgacagca gcctggaagc ctcggcagga cccctggggt gttgcagggt actagccgcc
tgcttgcgaa acccagcgtt cttcaggaga tgatgtgatg gaacacacac acacgcagac
acacacacac acacaaatgc agacacacaa catcaagaaa acagcaaggg agagaatcca

FIG. 2A

agcctaaaat taaataaatc ttccagcctg cttcttcccc agggttctgt attgcagcta
agctcaaatg tatatttaac ttctagttgc tcttgctttg gtcttcttcc aatgatgctt
actacagaaa gcaaatcaga cacaattaga gaa
(SEQ ID NO: 4)

FIG. 2B

MSLHFLYYCSEPTLDVKIAFCQGFDKQVDVSYIAKHYNMSKSKVDNQFY SVEVG DSTFTVL
KRYQNLKPIGSGAQGI VCAAYDAVLDRNVAIKKLSRPFQNO THAKRAYREL VLMKCVNHKN
IISLLNVFTPQKTLEEFQDVYLVME LMDANLCQVIQMEL DHERMSYLLYQMLCGIKHLHSA
GIIHRDLKPSNIVVKSDCTLKILDFGLARTAGTSFMMTPYVVTRY YRAPEVILGMGYKENV
DIWSVGCIMGEMVRHKILFPGRDYIDQWNKVIEQLGTPCPEFMKKLQPTVRNYVENR PKYA
GLTFPKLFPDSLFPADSEHNKLKASQARDLLSKMLVIDPAKRISVDDALQHPYINVWYDPA
EVEAPPPQIYDKQLDEREHTIEEWKELIYKEVMNSEEKTKNGVVKGQPSPSAQVQQ
(SEQ ID NO: 5)

FIG. 2C

GAGAAATGGCGTGGCAGGGGACCCAGCGAGCCCAGAGGGATTTTGCCGCTGCTTCCTCTAC
CCCTGTATTTACGCGAGCTCTCTAAATTGACTCAGCTCCAGGCTAGTGTGAGAAACACCAA
CAGCAGGCCCATCTCAGATCTTCACTATGGCAACTTATGCAAGAACTGTTGAATTAGACC
CGTTTCCTATAGATGAGAAACCATACAAGCTGTGGTATTTATGAGCCTCCATTTCTTATAC
TACTGCAGTGAACCAACATTGGATGTGAAAATTGCCTTTTGTGAGGGATTTCGATAAACAAAG
TGGATGTGTCATATATTGCCAAACATTACAACATGAGCAAAAGCAAAGTTGACAACCAGTT
CTACAGTGTGGAAGTGGGAGACTCAACCTTCACAGTTCTCAAGCGCTACCAGAATCTAAAG
CCTATTGGCTCTGGGGCTCAGGGCATAGTTTGTGCGCGTATGATGCTGTCCTTGACAGAA
ATGTGGCCATTAAGAAGCTCAGCAGACCCTTTCAGAACCAAACACATGCCAAGAGAGCGTA
CCGGGAGCTGGTCTCATGAAGTGTGTGAACCATAAAAACATTATTAGTTTATTAAATGTC
TTCACACCCCAGAAAACGCTGGAGGAGTTCCAAGATGTTTACTTAGTAATGGAAGTATGG
ATGCCAACTTATGTCAAGTGATTCAGATGGAATTAGACCATGAGCGAATGTCTTACCTGCT
GTACCAAATGTTGTGTGGCATTAAAGCACCTCCATTCTGCTGGAATTATTCACAGGGATTTA
AAACCAAGTAACATTGTAGTCAAGTCTGATTGCACATTGAAAATCCTGGACTTTGGACTGG
CCAGGACAGCAGGCACAAGCTTCATGATGACTCCATATGTGGTGACACGTTATTACAGAGC
CCCTGAGGTCATCCTGGGGATGGGCTACAAGGAGAACGTGGATATATGGTCTGTGGGATGC
ATTATGGGAGAAATGGTTCGCCACAAAATCCTCTTTCAGGAAGGGACTATATTGACCAGT
GGAATAAGGTAATTGAACAACTAGGAACACCATGTCCAGAATTCATGAAGAAATTGCAACC
CACAGTAAGAACTATGTGGAGAATCGGCCCAAGTATGCGGGACTCACCTTCCCCAAACTC
TTCCAGATTCCCTCTTCCCAGCGGACTCCGAGCACATAAACTCAAAGCCAGCCAAGCCA
GGGACTTGTTGTCAAAGATGCTAGTGATTGACCCAGCAAAAAGAATATCAGTGGACGACGC
CTTACAGCATCCCTACATCAACGTCTGGTATGACCCAGCCGAAGTGGAGGCGCCTCCACCT
CAGATATATGACAAGCAGTTGGATGAAAGAGAACACACAATTGAAGAATGGAAAGAACTTA
TCTACAAGGAAGTAATGAATTCAGAAGAAAAGACTAAAAATGGTGTAGTAAAAGGACAGCC
TTCTCCTTCAGCACAGGTGCAGCAGTGAACAGCAGTGAGAGTCTCCCTCCATCCTCGTCTG
TCAATGACATCTCCTCCATGTCCACCGACCAGACCCTGGCATCTGACACTGACAGCAGCCT
GGAAGCCTCGGCAGGACCCCTGGGTTGTTGCAGGTGACTAGCCGCCTGCCTGCGAAACCCA
GCGTTCTTCAGGAGATGATGTGATGGAACACACACACACGCGAGACACACACACACACAA
ATGCAGACACACAACATCAAGAAAACAGCAAGGGAGAGAATCCAAGCCTAAAATTAAATAA
ATCTTTCAGCCTGCTTCTTCCCCAGGGTTCTGTATTGCAGCTAAGCTCAAATGTATATTTA
ACTTCTAGTTGCTCTTGCTTTGGTCTTCTTCCAATGATGCTTACTACAGAAAGCAAATCAG
ACACAATTAGAGAAGCCTTTTCCATAAAGTGTAATTTTAATGGCTGCAAAACCGGCAACCT
GTAAGTGCCTTTTAAATGGCATGACAAGGTGTGCAGTGGCCCCATCCAGCATGTGTGTGT
CTCTATCTTGCATCTACCTGCTCCTTGGCCTAGTCAGATGGATGTAGATACAGATCCGCAT
GTGTCTGTATTATACAGCACTACTTACTTAGAGATGCTACTCTCAGTGTCTCAGGGCTC
TACCAAGACATAATGCACTGGGGTACCACATGGTCCATTTTCATGTGATCTATTACTCTGAC
ATAAACCCATCTGTAATATATTGCCAGTATATAAGCTGTTTGTAGTTTGTAAATTGATTAAAC
TGTATGTCTTATAAGAAAACATGTAAAGGGGGAATATATTGGGGGAGTGAGCTCTCTCAGA
CCCTTGAAGATGTAGCTTCCAAATTTGAATGGATTAAATGGCACCTGTATACCA

(SEQ ID NO: 6)

FIG. 2D

gagaaatggc gtggcagggg acccagcgag cccagagggg ttttgccgct gcttcctcta
ccccgtatt tcacgcagct ctctaaattg attcagctcc aggctagtgt gagaaacacc
aacagcaggg ccatctcaga tcttcactat ggcaacttat gcaagaaact gttgaattag
acccgtttcc tatagatgag aaaccataca agctgtggta tttatgagcc tccatttctt
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acaagtggat gtgtcatata ttgccaaaca ttacaacatg agcaaaaagca aagttgacaa
ccagttctac agtgtggaag tgggagactc aaccttcaca gttctcaagc gctaccagaa
tctaaagcct attggctctg gggctcaggg catagtctgt gccgcgtatg atgctgtcct
tgacagaaat gtggccatta agaagctcag cagacccttt cagaaccaa cacatgccaa
gagagcgtag cgggagctgg tctcatgaa gtgtgtgaac cataaaaaca ttattagttt
attaaatgtc ttcacacccc agaaaacgct ggaggagttc caagatgttt acttagtaat
ggaactgatg gatgccaaact tatgtcaagt gattcagatg gaattagacc atgagcgaat
gtcttacctg ctgtaccaaa tgttgtgtgg cattaagcac ctccattctg ctggaattat
tcacagggat ttaaaaccaa gtaacattgt agtcaagtct gattgcacat tgaaaatcct
ggactttgga ctggccagga cagcaggcac aagcttcatg atgactccat atgtggtgac
acgttattac agagccccctg aggtcatcct ggggatgggc tacaaggaga acgtggatat
atggctctgtg ggatgcatta tgggagaaat ggttcgccac aaaatcctct ttcagggaag
ggactatatt gaccagtggg ataaggtaat tgaacaacta ggaacaccat gtccagaatt
catgaagaaa ttgcaaccca cagtaagaaa ctatgtggag aatcggccca agtatgctgg
actcaccttc cccaaactct tcccagattc cctcttccca gcggactccg agcacaataa
actcaaagcc agccaagcca gggacttggt gtcaaagatg ctagtgattg acccagcaaa
aagaatatca gtggacgacg ccttacagca tccctacatc aacgtctggt atgacccagc
cgaagtggag gcgcctccac ctcagatata tgacaagcag ttggatgaaa gagaacacac
aattgaagaa tggaaagaac ttatctacaa ggaagtaatg aattcagaag aaaagactaa
aaatgggtgta gtaaaaggac agccttctcc ttcagcacag gtgcagcagt gaacagcagt
gagagtctcc ctccatcctc gtctgtcaat gacatctcct ccatgtccac cgaccagacc

FIG. 3A

ctggcatctg acactgacag cagccctggaa gcctcggcag gacccctggg ttgttgcagg
tgactagccg cctgcctgcg aaaccacagcg ttcttcagga gatgatgtga tggaacacac
acacacgcag acacacacac acacacaaat gcagacacac aacatcaaga aaacagcaag
ggagagaatc caagcctaaa attaaataaa tctttcagcc tgcttcttcc ccagggttct
gtattgcagc taagctcaaa tgtatattta acttctagtt gctcttgctt tggctcttct
ccaatgatgc ttactacaga aagcaaatac gacacaatta gagaagcctt ttccataaag
tgtaatttta atggctgcaa aaccggcaac ctgtaactgc ccttttaaat ggcattgacaa
gggtgtgcagt ggcccatcc agcatgtgtg tgtctctatc ttgcattctac ctgctccttg
gcctagtcag atggatgtag atacagatcc gcatgtgtct gtattcatac agcactactt
acttagagat gctactctca gtgtcctcag ggctctacca agacataatg cactggggta
ccacatggtc catttcatgt gatctattac tctgacataa acccatctgt aatatattgc
cagtatataa gctgttttagt ttgttaattg attaaactgt atgtcttata agaaaacatg
taaaggggga atatattggg ggagtgagct ctctcagacc cttgaagatg tagcttccaa
atattgaatgg attaaatggc acctgtatac ca

(SEQ ID NO: 7)

FIG. 3B

MSLHFLYYCSEPTLDVKIAFCQGFQVDVSYIAKHYNMSKSKVDNQFYSSVEVGDSF
TVLKRYQNLKPIGSGAQGIVCAAYDAVLDRNVAIKKLSRPFQNTAKRAYRELVLTK
CVNHKNIISLLNVFTPQKTLEEFQDVYLVMEMLDANLCQVIQMELDHERMSYLLYQML
CGIKHLHSAGIIHRDLKPSNIVVKSCTLKILDFGLARTAGTSFMMTPYVVTRYRAP
EVILGMGYKENVDIWSVGCIMGEMVRHKILFPGRDYIDQWNKVIEQLGTPCPEFMKKL
QPTVRNYVENRPPKYAGLTFPKLFPDSLFPADSEHNKLKASQARDLLSKMLVIDPAKRI
SVDDALQHPYINVWYDPAEVEAPPPQIYDKQLDEREHTIEEWKELIYKEVMNSEEKTK
NGVVKGQPSPSAQVQQ
(SEQ ID NO: 8)

FIG. 3C

CCCTCCTTATTCCGGTTTGGGAATGTGGCTAATGAAAGCCCAGTAGGAGGATTTCTGGGGCA
AACAGGTGGACCAGGATCCTGGTTCTCAGGCACGGAATGGCTATTGTGAGAGCGCCACCAG
CAGGACCATCGCAGATCTTGGTTATGGCTGCTCACGCAAGAGGCTGTTGATGTAGACCCCC
TTTCCCGTAGATGAGAAATCACACGAGCAGTGGTATTTATGAGCCTCCATTTCTTATACTA
CTGCAGTGAACCAACCTTGGATGTGAAAATTGCCTTTTGTGAGGTGTGTGTTCTTACAGG
TAAAACAAAGGGATTTCGACAAACACGTGGATGTGTCTTCTGTTGTCAAACATTACAACATG
AGCAAAGCAAGGTAGATAACCAGTTCTACAGTGTGGAAGTGGGAGACTCAACCTTCACAG
TTCTAAAGCGCTACCAGAACCTGAAGCCGATCGGCTCTGGGGCTCAGGGAATAGTTTGTGC
TGCATATGACGCTGTCCTCGACAGAAATGTGGCCATTAAGAAGCTCAGCAGACCCTTCCAG
AACCAAACTCATGCCAAGAGGGGCTTACCGGGAGCTGGTCTCATGAAGTGTGTGAACCATA
AAAACATTATTAGCTTATTAAATGTCTTTACACCCAGAAAACACTGGAGGAGTTCCAAGA
TGTTTACTTAGTGATGGAACCTGATGGACGCCAACTTGTGTGAGGTGATTCAGATGGAGCTG
GACCACGAGCGGATGTCGTACTTGTGTACCAGATGCTGTGCGGCGATCAAACACCTCCACT
CCGCTGGGATCATCCACAGGGACTTAAAACCCAGTAACATCGTAGTCAAGTCTGATTGCAC
ACTGAAAATCCTGGACTTTGGACTGGCCAGGACAGCGGGCACAAGCTTCATGATGACTCCG
TATGTGGTGACGAGATATTACAGAGCCCCCGAGGTCATCCTGGGCATGGGCTACAAGGAGA
ACGTGGACATATGGTCTGTGGGCTGCATCATGGGAGAAATGGTTCGTCACAAATCCTCTT
TCCCGGAAGGGACTATATTGACCAGTGGAAACAAAGTCATAGAGCAGCTAGGAACTCCGTGT
CCAGAATTCATGAAGAAATTGCAGCCCACCGTCAGAACTACGTGGAGAACCGGCCCAAGT
ATGCAGGCCTCACCTTCCCCAAGCTCTTTCCAGATTCCCTCTTCCCAGCGGATTCGGAGCA
CAATAAACTTAAAGCCAGCCAAGCCAGGGACTTGTGTCAAAGATGTTAGTGATTGACCCA
GCGAAGAGGATATCGGTGGATGACGCATTGCAGCATCCGTACATCAACGTTTGGTACGACC
CTGCTGAAGTGGAGGCGCCTCCGCCTCAGATATATGACAAGCAATTGGATGAAAGGGAGCA
CACCATCGAAGAATGGAAAGAACTCATCTACAAGGAAGTAATGAACTCAGAAGAGAAGACT
AAGAACGGCGTAGTCAAAGGCCAGCCCTCACCTTCAGGTGCAGCAGTGAACAGCAGTGAGA
GTCTCCCTCCATCCTCATCTGTCAACGACATCTCCTCCATGTCCACCGACCAGACCCTCGC
ATCCGACACTGACAGCAGCCTGGAAGCCTCGGCGGGACCGCTGGGTTGTTGCAGGTGACTA
GCCGCCTGCCTGCGAAACCCAGCGTTCTTCAGGAGATGACGCCATGATAGAACACAGCGCA
CATGCACACACACAGAGCTTGTACACACACACACACACACACACACACGACGACGACGACG
CACGCAAGCACGCACGCACGCACAAATGCACTCACGCAATGTCAAGAAAAAAAAGTAGC
GAGAGAGAGCGAGAGAGCCAACGTAAACTAAGTTAAATCTTTCTGCGTGCTTCTCCAGAG
TTCTGTATCGCAGCTGAGCTGAAATGTATACTTAACCTTAGTCGCGCTCGCTCGACTTTG
GTCTCCCTCCGGCAGTGCTTACT

(SEQ ID NO: 9)

FIG. 4A

MSKSKVDNQFY SVEVG DSTFTVLKRYQNLKPIGSGAQGI VCAAYDAVLDRNVAIKKLSRPF
QNQTHAKRAYREL VLMKCVNHKNIISLLNVFTPQKTLEEFQDVYLVME LMDANLCQVIQME
LDHERMSYLLYQMLSAIKHLHSAGIIHRDLKPSNIVVKS DCTLKILDFGLARTAGTSFMMT
PYVVTRY YRAPEVILGMGYKENVDIWSVGCIMGEMVRHKILFPGRDYIDQWNKVIEQLGTP
CPEFMKKLQPTVRNYVENRPKYAGLTFFPKLFPDSLFPADSEHNK LKASQARDLLSKMLVID
PAKRISVDDALQHPYINVWYDPAEVEAPPPQIYDKQLDEREHTIEEWKELIYKEVMNSEEK
TKNGVVKGQPSPSGA AVNSSES LPPSSSVNDISSMSTDQTLASDTDS SLEASAGPLGCCR
(SEQ ID NO:10)

FIG. 4B

GGGGCTTGAGTGAGCTAAAGATTGGGTCTTCTTGGAAATCACCTGTCTGTTATTATTTTAA
 AACAAATCGCTACACCTCCAAAGACTCTGCTCCTTACTCCGGTTTGGAAATGTGGCTAATGAC
 TACCCAGTAGGGAGGATTTCTGGGGCAAACAGCCGGACCAGGATCCTAGTTCTCAGGCACG
 GAATGGCTATTGTGAGAACAGCACCAGCAGGATCATCGCAGATCTTGGTTATGGCCACTCA
 GGCAAGACGCTGTTGAGTTAAGACCCCTTTCCCATAGATGAGAAGCCACAGAAGCAGTGGT
 ATTTATGAGCCTCCATTTCTTATACTACTGCAGTGAACCAACCTTGGATGTGAAAATTGCC
 TTTTGTGTCAGGGATTTCGATAAACACGTGGATGTGTGTCATCTATTGCCAAACATTACAACATGA
 GCAAAAGCAAGGTGGACAACCAGTTCTACAGTGTGGAAGTGGGGGACTCAACCTTCACCGT
 TCTTAAGCGCTACCAGAACCTGAAGCCAATTGGCTCTGGGGCTCAGGGAATAGTCTGTGCT
 GCGTACGACGCTGTCCTTGACAGAAATGTGGCCATTAAGAAGCTCAGCAGACCCTTCCAGA
 ACCAAACTCACGCCAAGAGGGCTTACCGGGAGCTGGTGCTCATGAAGTGTGTGAACCATAA
 AACATTATTAGCTTATTAAATGTTTTTACACCCCAAGAAACGCTGGAGGAGTTCCAAGAT
 GTCTACTTAGTGATGGAACCTGATGGACGCCAACCTGTGTGTCAGGTGATTCAGATGGAGCTGG
 ACCACGAGCGGATGTCTTACTTGCTGTACCAGATGCTGTGTGGCATCAAGCACCTCCACTC
 CGCTGGGATCATCCACAGGGACTTAAAACCCAGTAACATTGTAGTCAAGTCTGATTGCACA
 CTGAAAATCCTCGACTTCGGACTGGCCAGGACAGCGGGTACAAGCTTCATGATGACTCCGT
 ATGTGGTGACGCGATATTACAGAGCCCCTGAGGTTCATCCTGGGCATGGGCTACAAGGAGAA
 CGTGGACATATGGTCTGTGGGATGCATCATGGGAGAAATGGTTCGCCACAAAATCCTCTTT
 CCCGGAAGGAGCTATATTGACCAGTGGAACAAAGTCATCGAGCAGCTAGGAACTCCGTGTC
 CAGAGTTCATGAAGAAATTGCAGCCACAGTCAGAAACTACGTGGAGAATCGGCCCAAGTA
 CGCAGGACTCACCTTCCCCAAGCTCTTTCAGATTCCCTCTTCCCAGCGGATTCTGAGCAC
 AATAAACTTAAAGCCAGCCAAGCCAGGGATTTGTTGTCTAAGATGTTAGTGATTGACCCAG
 TGAAGAGGATATCGGTGGACGACGCACTGCAGCATCCGTACATCAACGTTTGGTACGACCC
 GGCTGAAGTGGAGGCGCCTCCGCCTCAGATATATGATAAGCAGCTGGATGAAAGGGAGCAC
 ACCATCGAAGAATGGAAAGAACTTATCTACAAGGAGGTAATGAACTCAGAAGAGAAGACTA
 AGAATGGCGTAGTCAAAAGCCAGCCCTCGCCTTCAGCACAGGTGCAGCAGTGAACAGCAGT
 GAGAGTCTCCCTCCATCCTCGGCTGTCAACGACATCTCCTCCATGTCCACCGACCAGACCC
 TCGCATCTGACACTGACAGCAGCCTGGAGGCCTCGGCGGGACCGTTGGGTTGTTGCAGGTG
 ACTAGCCGCCTGCCTGCGAAACCCAGCGTTCTTCAGGAGATGACGCGATAGAACACAGCAC
 ACATGCACACACACAGCTTGCTCTCACACACACTCAGCTTGCTCACACACACACACACA
 TACACACAAACACACACTGTCTCTCTCTCACACACACACACTGTCAACGCACTCACGAA
 AGGTCAAGAAAAAATAACAATAGAGAGATCCAACATAAAATTAAGTTAAATTTTTCTGCG
 TGCTTCTCCAAAGTTCTGTATCACAGCTGAGCTGAAATGTATACTTAAGTTAAATTTTTCTGCG
 TCGCTTTGGTTTCCCTCCAGCAGTGCTTACTACACAAGACAAATCAGACACAATTAGAGAA
 ACCTTTCCCTAAAGTGTAAGTTAAGTGGCTGCAGAACCAGCAACCTGTAAGTGCCTTCAA
 ATGGCATGAGGAGGTGGGCACGGGTCCCGCCCAGCATGTGTGTGTCTCTATCTCGCGTCTA
 CCTGCTCTTCCGGCCTAGTCAGATGGATGTAGATACAGATCCCGCATGTGTCTGTATTCAA
 ACAGCACTTAGAGATGCTCCTGTGAGTGTCTCCAGGCTCCACCAAGACACACACCGGGGT
 ACCACATGGTCCATTTTCATGTGATCTATTACTCTGACATAAATCCATCTGTAATATATTGC
 CAGTATATAAGCTGTTTAGTTTGTAAATTGCTTAAGCTGTATGTCTTATAAGAGACTATGT
 AAAGGGGGAAATGGAGGCGTGAAGTCTCAGACCCTTGAAGATGTAGCTTCCGAATTTGAC
 CGTTAAATGGCACCGTATACC

(SEQ ID NO:11)

FIG. 5A

MSLHFLYYCSEPTLDVKIAFCQGFDKHVDVSSIAKHYNMSKSKVDNQFY SVEVGDSTFTVL
KRYQNLKPIGSGAQGIVCAAYDAVLDRNVAIKKLSRPFQNTAKRAYRELVLMKCVNHKN
IISLLNVFTPQKTLEEFQDVYLVMEMLMDANLCQVIQMELDERMSYLLYQMLCGIKHLHSA
GIIHRDLKPSNIVVKSDCTLKILDFGLARTAGTSFMMTPYVVTRYRRAPEVILGMGYKENV
DIWSVGCIMGEMVRHKILFPGRSYIDQWNKVIEQLGTPCPEFMKKLQPTVRNYVENRPKYA
GLTFPKLFPDSLFPADSEHNKLKASQARDLLSKMLVIDPVKRISVDDALQHPYINVWYDPA
EVEAPPPQIYDKQLDERHTIEEWKELIYKEVMNSEKTKNGVVKSQPSPSAQVQQ

(SEQ ID NO:12)

FIG. 5B

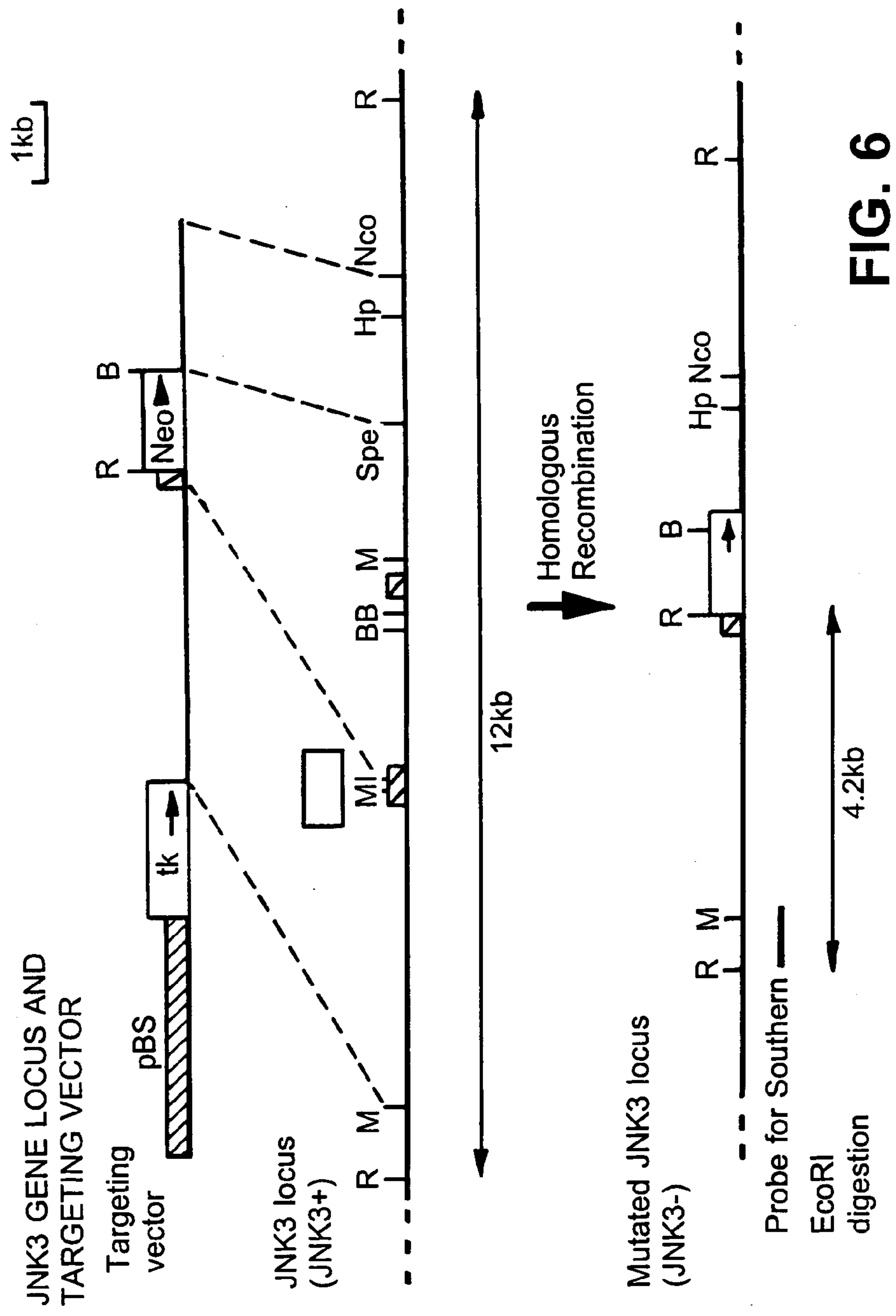


FIG. 6

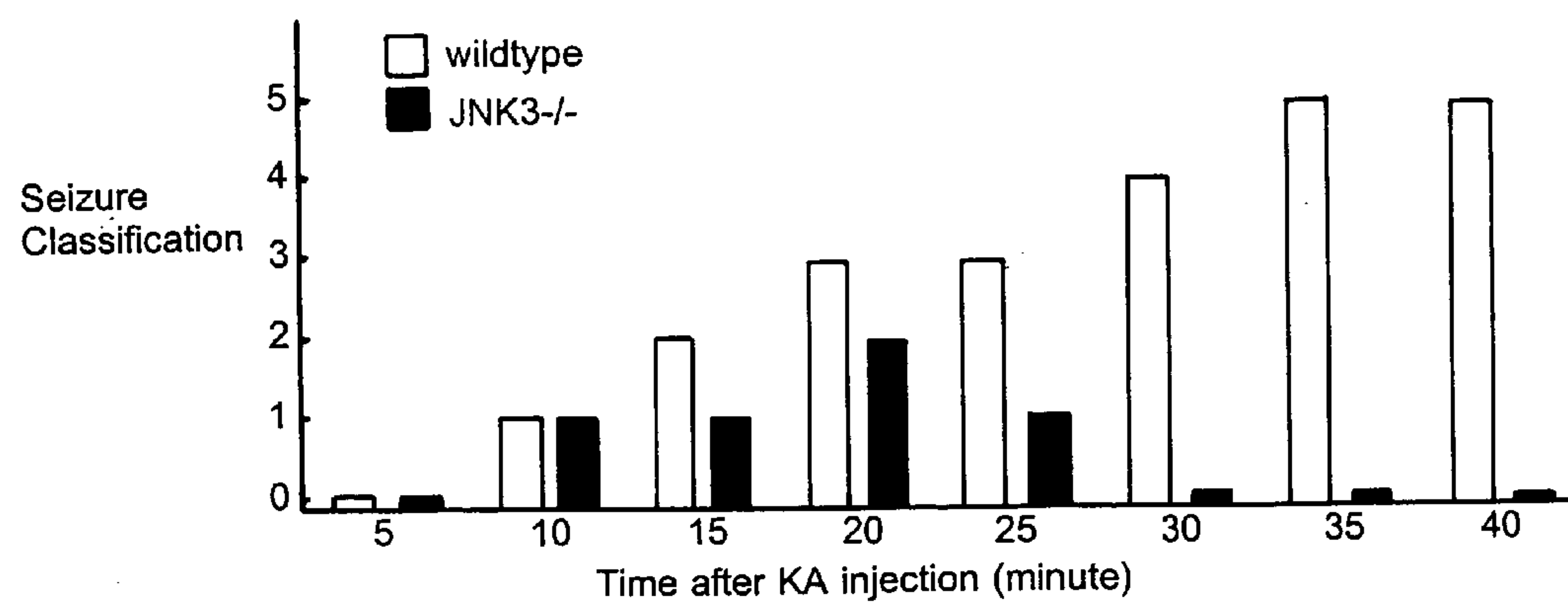


FIG. 7

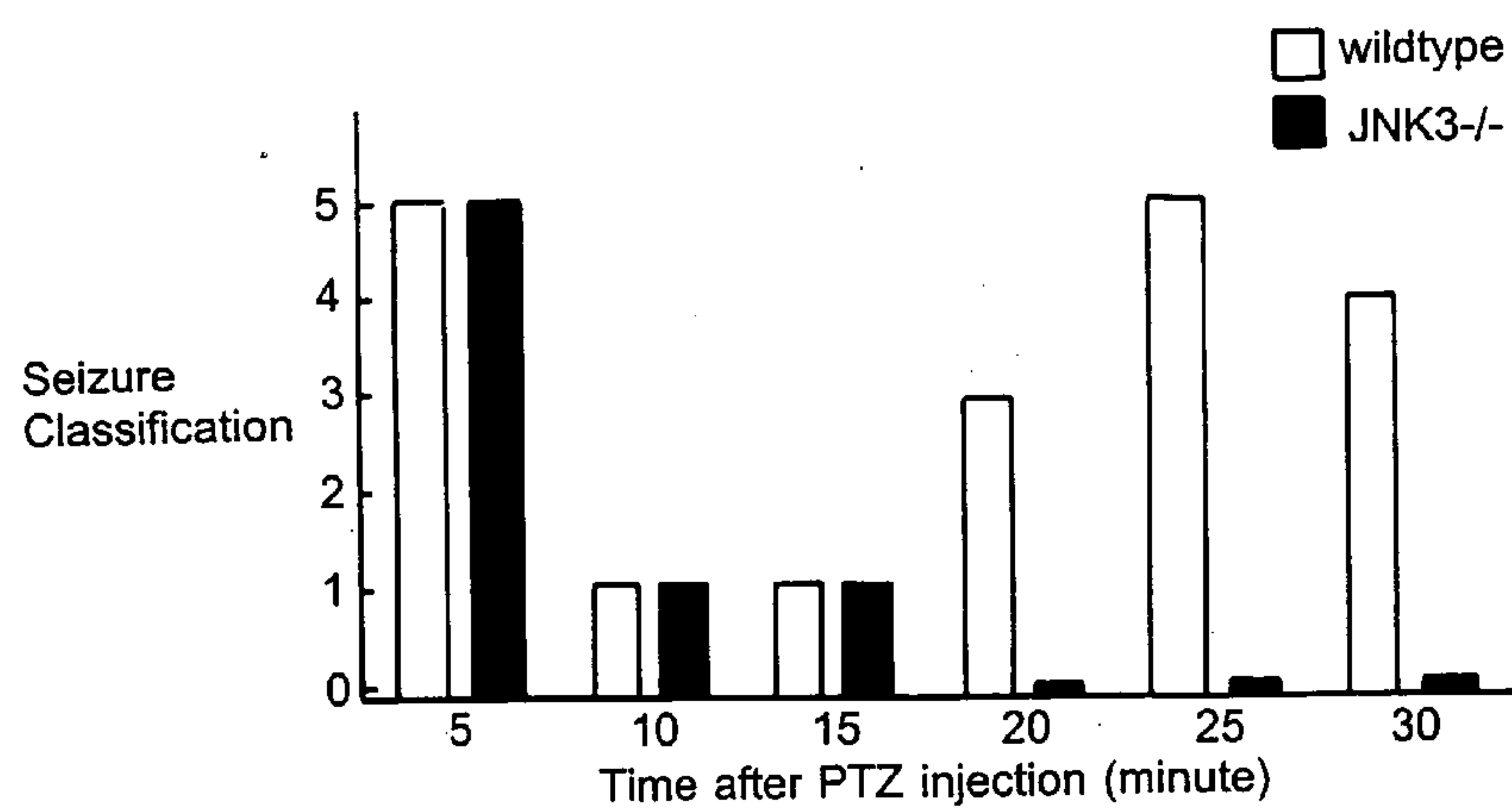


FIG. 8

agcaacttttc ctgacccaga ggaccggtaa caagtggccg ggagcaactt ttgcaaactct
cttctgcgcc ttaaggctgc caccgagact gtaaagaaaa ggagaagagg aacctatact
cataccagtt cgcacaggcc taagttagggc gaggcctagc cgcggctgcc tagcgtcccc
ccccccctca cagcggagga ggggacagtt gttggaggcc gggcggcaga cccgatcgcg
ggcctccacc gagaattccg tgacgactgg tcagcaccgc cggagagccg ctggttgcctg
gactgggtctg cgggctccaa ggaaccgctg ctccccgaga gcgctccgtg agtgaccgcg
acttttcaaa gctcggcatc gcgcggagtc ctaccaacgt gagtgcctagc ggagtcctta
ccctgcgctc cctggagcga actggggagg agggctcagg gggaagcact gccgtctgga
gcgcacgctc taaacaaact ttgttacaga agcagggacg cgcgggtatc cccccgett
ccggcgcgct gttgcggccc cgaaacttct gcgcacagcc caggctaacc ccgctgaag
tgacggaccg ttctatgact gcaaagatgg aaacgacctt ctacgacgat gccctcaacg
cctcgttcct ccagtccgag agcggtgccct acggctacag taaccctaag atcctaaaac
agagcatgac cttgaacctg gccgaccggt tgggcagctc gaagccgcac ctccgcgcca
agaactcggg ccttctcacg tcgcccagcg tcgggctgct caagctggcg tcgccggagc
tgagagcgct gatcatccag tccagcaatg ggcacatcac cactacaccg acccccaccc
agttcttctg ccccaagaac gtgaccgacg agcaggaggg cttcgccgag ggcttcgtgc
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tcaaccggg tgcgctgagc agcggcggtg gggcgccctc ctatggcgcg gccgggctgg
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cgctgtgccg cgcgcgacca gaacgatgga cttttcgta acattgacca agaactgcat
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ttgtaaataa gagatttgaa gcattctgag tttaccatth gtaataaagt atataatttt
tttatgtttt gtttctgaaa atttccagaa aggatattht agaaaatata ataaactatt
gaaaagt

(SEQ ID NO:13)

FIG. 9A

MTAKMETTFYDDALNASFLQSESGAYGYSNPKILKQSMTLNLAD
PVGSLKPHLRAKNSDLLTSPDVGLLKLASPELERLI IQSSNGHITTTPTPTQFLCPKN
VTDEQEGFAEGFVRALAE LHSQNTLPSVTSAAQPVSGAGMVAPAVASVAGAGGGGGYS
ASLHSEPPVYANLSNFNPGALSSGGGAPSYGAAGLAFPSQPQQQQQPPQPPHHLPQQI
PVQHPR LQALKEEPQTVPEMPGETPPLSPIDMESQERIKAE RKMRNR IAASKCRKRK
LERIARLEEKV KTLKAQNSELASTANMLREQVAQLKQKVMNHVNSGCQLMLTQQLQTF
(SEQ ID NO: 14)

FIG. 9B

ataccagaga	ctcaaaaaaa	aaaaaaaaag	tccagattgc	tggacaatga	cccggggtctc
atcccttgac	cctgggaacc	gggtccacat	tgaatcaggt	gcgaatgttc	gctcgccttc
tctgcctttc	ccgcctcccc	tcccccgcc	gcggecccg	ttccccccct	gcgctgcacc
ctcagagtgt	gctgcagccg	gcgagctgtt	cccgtcaatc	cctccctcct	ttacacagga
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tgtgaacact	cataggtgaa	agatgtatgc	caagacgggg	gttgaaagcc	tggggcgtag
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gcccagtgac	gtaggaagtc	catccattca	cagcgcttct	ataaaggcgc	cagctgaggc
gcctactact	ccaaccgcga	ctgcagcgag	caactgagaa	gactggatag	agccggcggt
tccgcgaacg	agcagtgacc	gcgctcccc	ccagctctgc	tctgcagctc	ccaccagtgt
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accttaactg	aatgttggtc	cttttcttct	atagctatct	cctgaagagg	aagagaaacg
gagaatccga	agggaacgga	ataagatggc	tgcagccaag	tgccggaatc	ggaggaggga
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agttggagct	tgggactatg	ggcgcagggt	ccttgagcat	gcccgtgtct	tatgctttct
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tgagattgcc	aatctgctga	aagagaagga	aaaactggag	tttattttgg	cagcccaccg
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tgtcttcacc	tacctgaag	ctgactcctt	cccaagctgt	gccgctgccc	accgaaagg
cagcagcagc	aacgagccct	cctccgactc	cctgagctca	cccacgctgc	tggccctgtg

FIG. 10A

agcagtcaga gaaggcaagg cagccggcat ccagacgtgc cactgcccga gctgggtgcat
tacagagagg agaaacacgt cttccctcga aggttcccgt cgacctaggg aggaccttac
ctgttcgtga aacacaccag gctgtgggccc tcaaggactt gcaagcatcc acatctggcc
tccagtcctc acctcttcca gagatgtagc aaaaacaaaa caaaacaaaa caaaaaaccg
catggagtgt gttgttccta gtgacacctg agagctggta gttagtagag catgtgagtc
aaggcctggc ctgtgtctct tttctctttc tccttagttt tctcatagca ctaactaatc
tgttgggttc attattggaa ttaacctggc gctggattgt atctagtgcg gctgatttta
acaataccta ctgtgttcct ggcaatagcg tgttccaatt agaaacgacc aatattaaac
taagaaaaga taggacttta ttttccagta gatagaaatc aatagctata tccatgtact
gtagtccttc agcgtcaatg ttcattgtca tgttactgat catgcattgt cgagggtggc
tgaatgttct gacattaaca gttttccatg aaaacgtttt tattgtgttt tcaatttatt
tattaagatg gattctcaga tattttatac tttattttat ttttttctac cctgaggtct
ttcgacatgt ggaaagtga tttgaatgaa aaattttaag cattgtttgc ttattgttcc
aggacattgt caataaaaagc atttaagttg aatgcgacca ccttcttgct ctctttattc
tcagttt (SEQ ID NO:15)

FIG. 10B

MMFSGFNADYEASSSRCSSASPAGDSLSEYHSPADSFSSMGSPV
NTQDFCADLSVSSANFIPTVTAISTSPDLQWLVOPTLVSSVAPSQTRAPHYPYGLPTQS
AGAYARAGMVKTVSGGRAQSIGRRGKVEQLSPEEEKRRIRRRERNKMAAAKCRNRRRE
LTDTLQAETDQLEDEKSALQTEIANLLKEKEKLEFILAAHRPACKIPDDLGFPEEMSV
ASLDLTGGLPEASTPESEEAFTLPLLNDPEPKPSLEPVKSISNVELKAEPFDDFLFPA
SSRPSGSETSRSPDVLDSGSFYAADWEPLHSNSLGMGPMVTELEPLCTPVVTCTPGC
TTYTSSFVFTYPEADSFPSCAAHRKGSSSNEPSSDSLSSPTLLAL
(SEQ ID NO:16)

FIG. 10C

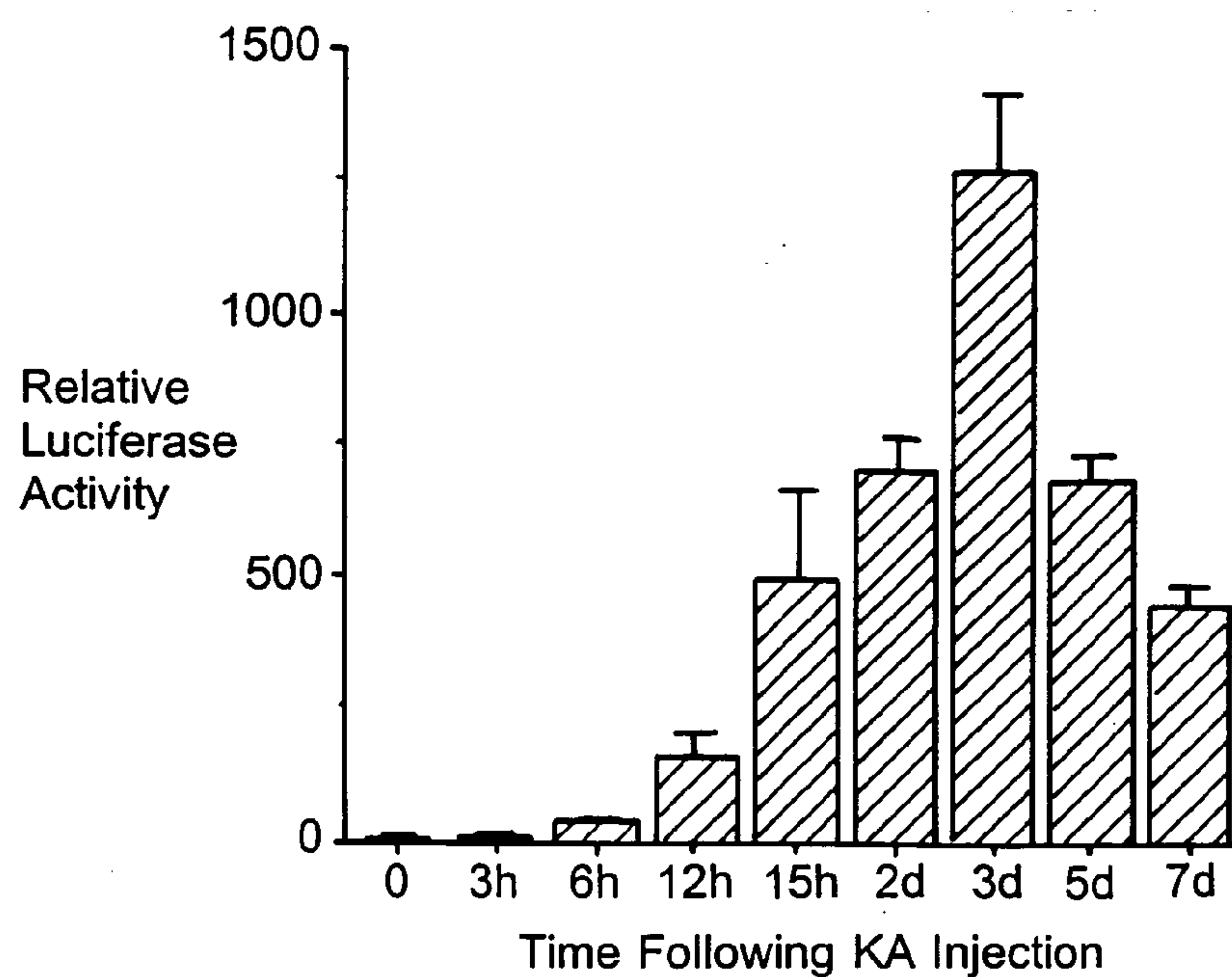


FIG. 11

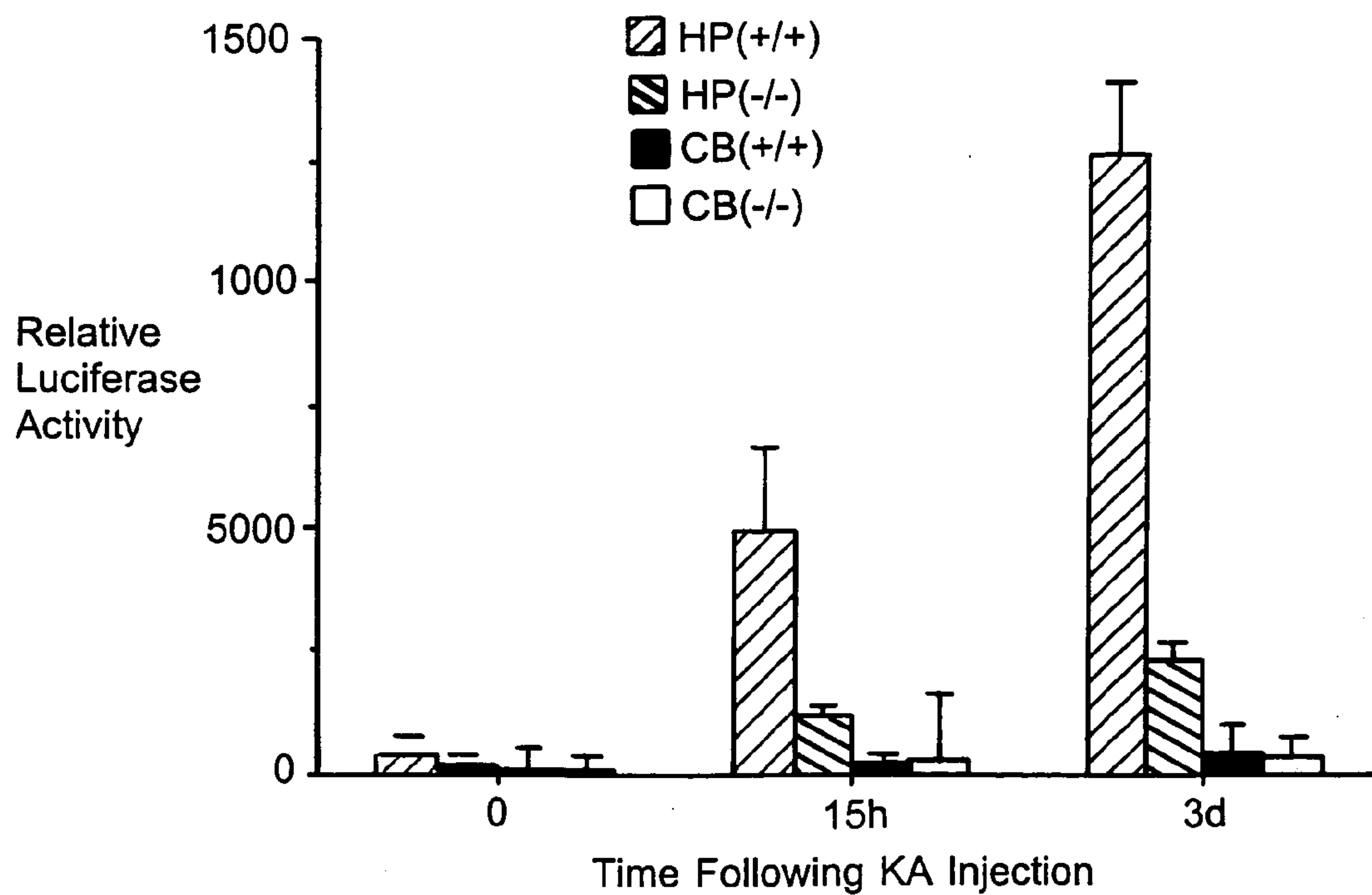


FIG. 12

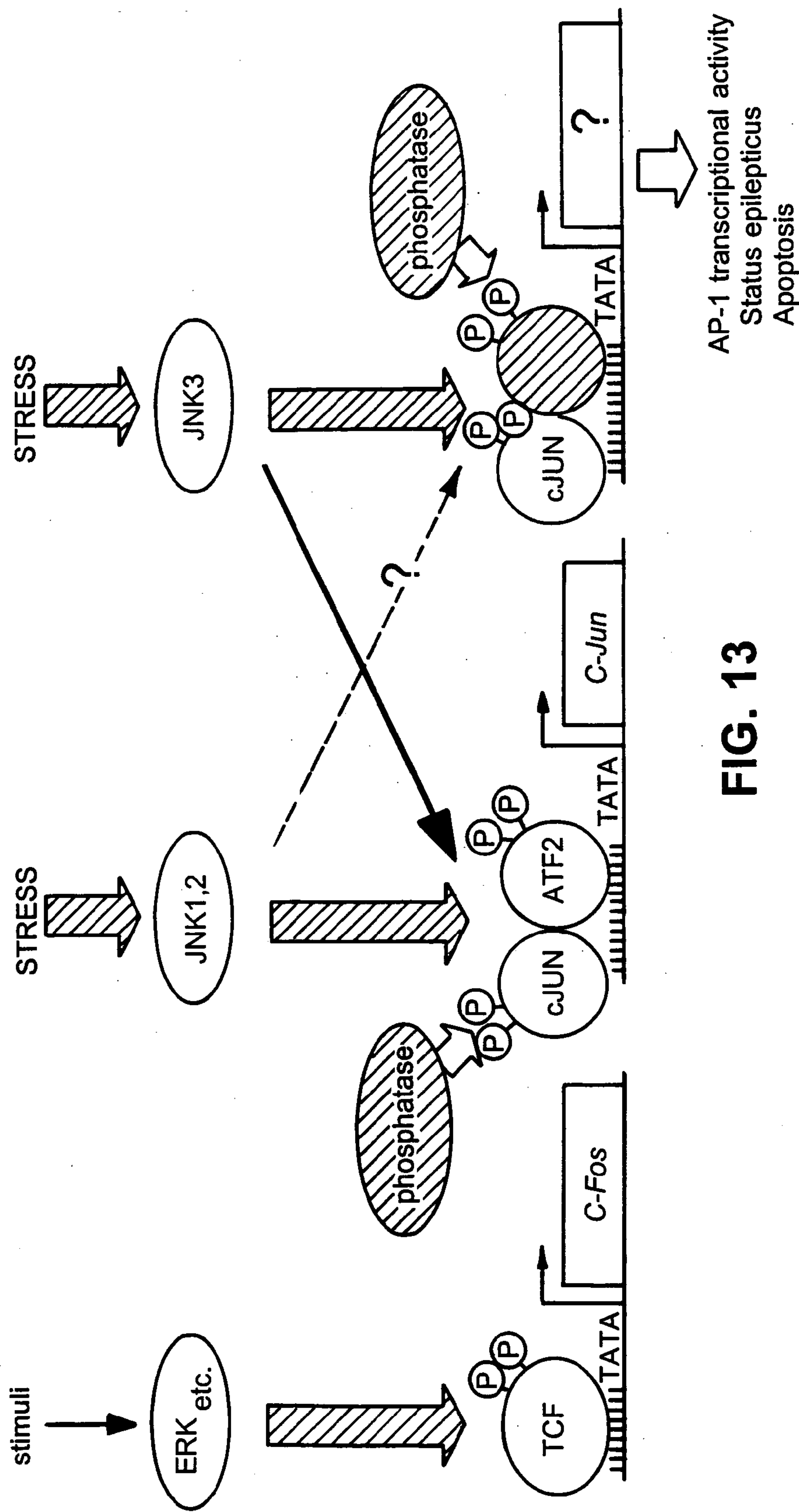


FIG. 13

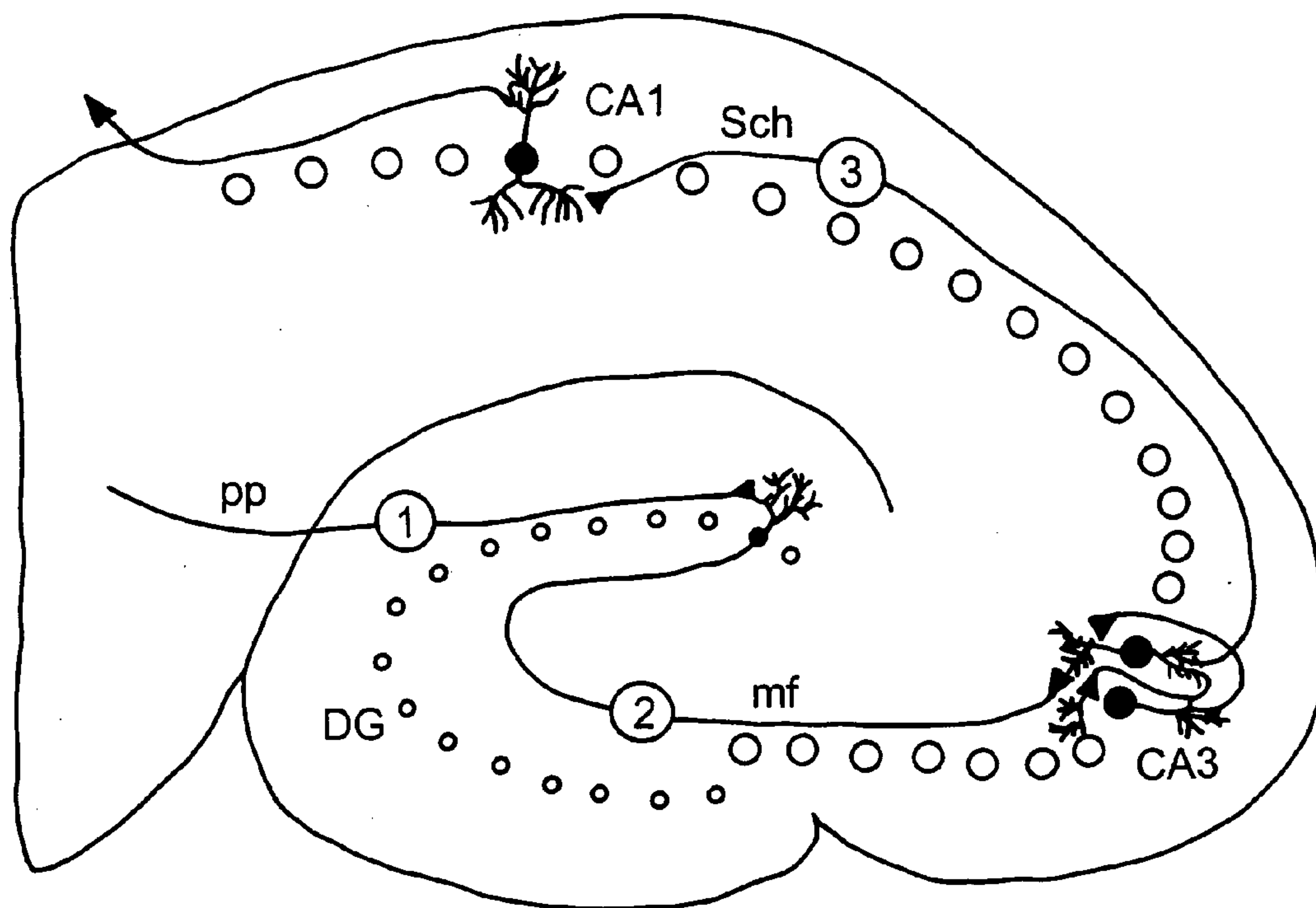


FIG. 14

JNK3 MODULATORS AND METHODS OF USE

[0001] This application claims priority from provisional application Ser. No. 60/060,995, filed Oct. 3, 1997.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made, in part, with support from the National Institutes of Health. The government may have certain rights in the invention.

FIELD OF THE INVENTION

[0003] The invention relates to screening assays for the detection of inhibitors of protein kinase expression or activity.

BACKGROUND OF THE INVENTION

[0004] Apoptosis, or programmed cell death, is a prominent feature of the nervous system during normal development and in adult brain exposed to environmental stress (Kuida et al., *Nature*, 384:368-372, 1996; Ratan et al., *Neurochem.*, 62:376-379, 1994; Raff et al., *Science*, 262:695-700, 1993). Stress-induced apoptosis has been implicated in a variety of neurological diseases (Thompson, *Science*, 267:1456-1462, 1995) and requires de novo protein and RNA synthesis (Martin et al., *J. Cell. Biol.*, 106:829-844, 1988; Oppenheim et al., *Dev. Biol.*, 138:104-113, 1990). Increased expression of c-Jun protein is associated with neuronal damage following global ischemia (Neumann-Haefelin et al., *Cerebral Flow Metab.*, 14:206-216, 1994) or transection of nerve axons in vivo (Neumann-Haefelin, *supra*). Increased expression and phosphorylation of c-Jun have been observed in vitro prior to the apoptotic death of sympathetic neurons deprived of nerve growth factor (NGF) (Ham et al., *Neuron*, 14:927-939, 1995). Moreover, expression of a dominant negative mutant c-Jun, or treatment with c-Jun antibody protects NGF-deprived sympathetic neurons from apoptosis (Ham et al., *supra*; Estus et al., *J. Cell. Biol.*, 127:1717-1727, 1994). However, the requirement of c-Jun for stress-induced neuronal apoptosis has not been tested in vivo since c-Jun deficient mice die during mid-gestation (Hilberg et al., *Nature*, 365:179-181, 1993).

[0005] Protein phosphorylation is one important mechanism involved in the activation of c-Jun in response to environmental stress signals (Whitmarsh et al., *J. Mol. Med.*, 74:589-607, 1996). c-Jun N-terminal kinase (JNK, also known as SAPK) is a serine/threonine protein kinase that phosphorylates two residues (Ser-63 and Ser-73) on the NH₂-terminal activation domain of c-Jun (Whitmarsh et al., *supra*; Dèrijard et al., *Cell*, 76:1025-1037, 1994; Kyriakis et al., *Nature*, 369:156-160, 1994). Map kinase kinase (MKK) 4 (also known as SEK1) is a direct activator of JNK in response to environmental stresses and mitogenic factors (Whitmarsh et al., *supra*; Dèrijard et al., *supra*; Nishina et al., *Nature*, 385:350-353, 1997; Yang et al., *Proc. Nat. Acad. Sci. USA*, 94:3004-3009, 1997; Sanchez et al., *Nature*, 372:794-798, 1994). JNK also phosphorylates ATF2 and other Jun-family proteins which function as components of the AP-1 transcription factor complex (Whitmarsh et al., *supra*; Gupta et al., *Science*, 267:389-393, 1995; Gupta et al., *EMBO J.*, 15:2760-2770, 1996). The phosphorylation of these transcription factors by JNK leads to increased AP-1 transcrip-

tional activity (Whitmarsh et al., *supra*). Conversely, the induction of AP-1 transcriptional activity is selectively blocked in cells lacking MKK4 (Yang et al., *supra*).

[0006] JNK has been implicated in the apoptosis of NGF-differentiated PC12 pheochromocytoma cells (Xia et al., *Science*, 270:1326-1311, 1995), one model system of neuronal cell death in vivo (Batistatou et al., *J. Cell. Biol.*, 122:523-532, 1993). When differentiated PC12 cells are deprived of nerve growth factor (NGF), JNK activation is observed prior to apoptotic death (Xia et al., *supra*). Transfection studies using constitutively activated and dominant negative mutant components of the JNK signaling pathway established that JNK is involved in NGF withdrawal-induced apoptosis of PC12 cells (Xia et al., *supra*).

[0007] Ten JNK isoforms, resulting from alternative splicing of three different genes have been identified (Dèrijard et al., *supra*; Kyriakis et al., *supra*; Gupta et al., *supra*; Martin et al., *Brain Res. Mol. Brain Res.*, 35:47-57, 1996). Although the JNK1 and JNK2 isoforms are widely expressed in murine tissues, including the brain, the JNK3 isoforms are predominantly expressed in the brain and, to a lesser extent, in the heart and testis.

SUMMARY OF THE INVENTION

[0008] The invention is based on the discovery that mice lacking the JNK3 gene (JNK3(-/-)) develop normally and are resistant to excitotoxic damage, and that JNK3 plays a role in stress-induced seizure activity, AP-1 transcriptional activation, and kainate-induced apoptosis of hippocampal neurons. Thus, JNK3 is a mediator of kainate/glutamate excitotoxicity and a target for limiting or preventing excitotoxic damage.

[0009] The invention features a method of identifying a candidate compound that modulates JNK3 expression. The method includes the steps of incubating a cell that can express a JNK3 protein with a compound under conditions and for a time sufficient for the cell to express the JNK3 protein when the candidate compound is not present. The expression of JNK3 is then measured in the cell in the presence of the compound. The expression of JNK3 is also measured in a control cell under the same conditions and for the same time. The amount of JNK3 expression in the cell incubated in the presence of the compound and in the control cell is compared. A difference in JNK3 expression indicates that the compound modulates JNK3 expression. In an embodiment of this method, the compound decreases JNK3 expression.

[0010] In another embodiment, the invention features a method of identifying a candidate compound that modulates JNK3 activity. The method includes the steps of incubating a cell that has JNK3 activity with a compound under conditions and for a time sufficient for the cell to express JNK3 activity when the candidate compound is not present. The activity of JNK3 is then measured in the cell in the presence of the compound. The activity of JNK3 is also measured in a control cell under the same conditions and for the same time. The amount of JNK3 activity in the cell incubated in the presence of the compound and in the control cell is compared. A difference in JNK3 activity indicates that the compound modulates JNK3 activity. In an embodiment of this method, the compound decreases JNK3 activity.

[0011] The invention also includes a method of identifying a compound that modulates the binding of a JNK3 polypeptide to a substrate. The method involves comparing the amount of a JNK3 polypeptide bound to a substrate in the presence and absence of a selected compound. A difference in the amount of binding of a JNK3 polypeptide to the substrate indicates that the selected compound modulates the binding of a JNK3 polypeptide. In an embodiment of this method, the binding of a JNK3 polypeptide to a substrate is decreased.

[0012] Another feature of the invention is a method for generating a totipotent mouse cell comprising at least one inactivated JNK3 gene. The method includes: a) providing a plurality of totipotent mouse cells; b) introducing into the cells a DNA construct that includes a mouse JNK3 gene disrupted by the insertion of a sequence into the gene, thus the disruption prevents expression of functional JNK3; c) incubating the cells so that homologous recombination occurs between the chromosomal sequence encoding JNK3 and the introduced DNA construct; and d) identifying a totipotent mouse cell that has at least one inactivated JNK gene.

[0013] Also featured in the invention is a method for generating a mouse homozygous for an inactivated JNK3 gene. The method includes the steps of: a) providing a totipotent mouse cell that contains at least one inactivated JNK3 gene; b) inserting the cell into a mouse embryo and implanting the embryo into a female mouse; c) permitting the embryo to develop into a neonatal mouse; d) permitting the neonatal mouse to reach sexual maturity; e) mating two of the sexually mature mice to obtain a mouse homozygous for the inactivated JNK3 gene. Such a mouse (homozygous JNK3(-/-)) is resistant to excitotoxic damage.

[0014] The invention also features methods of treating a patient having or at risk for a disorder of the nervous system involving excitotoxicity. The methods include administering to the patient a therapeutically effective amount of a compound that inhibits JNK3 expression, or a therapeutically effective amount of a compound that inhibits JNK3 activity. An antisense nucleic acid molecule or ribozyme can be used as the inhibitory compound. Disorders that can be treated by these methods include dementias including Alzheimer's disease, neurodegenerative diseases such as Huntington disease, cerebrovascular disorders such as ischemia, amyotrophic lateral sclerosis, trauma including that caused by heat or cold, motor neuron disease, Parkinson's disease, or seizure disorders including epilepsy. Neuroendocrine disorders such as those that affect pituitary glands, adrenal glands, testis, or pancreas (e.g., β -cells) can be treated with JNK3 modulators.

[0015] The invention also includes a transgenic non-human mammal having a transgene disrupting expression of a JNK3 gene, the transgene being chromosomally integrated into germ cells of the mammal. In an embodiment of the invention, the mammal is a mouse. The germ cells of the mammal can be homozygous for the transgene and the disruption of JNK3 gene expression can be the result of a null mutation. Another embodiment of the invention includes a cell line descended from a cell of the mammal having the transgene disrupting expression of a JNK3 gene.

[0016] A DNA construct comprising a disrupted mouse JNK3 gene is also featured in the invention. The disruption

is by insertion of a sequence into the gene such that the disruption prevents or modifies the expression of functional JNK3.

[0017] Unless otherwise specified, "JNK3" can refer both to nucleic acids and polypeptides, such as the sequences shown in **FIGS. 1A-5B** (SEQ ID NOS:1-12; see also, GenBank accession number: U34819 which corresponds to SEQ ID NO:1 and SEQ ID NO:2; U34820 which corresponds to SEQ ID NO:4 and SEQ ID NO:5; U07620 which corresponds to SEQ ID NO:7 and SEQ ID NO:8; L27128 which corresponds to SEQ ID NO:9 and SEQ ID NO:10; and L35236 which corresponds to SEQ ID NO:11 and SEQ ID NO:12). SEQ ID NO:3 and SEQ ID NO:6 represent deduced nucleotide sequences based on the presumed overlap between the sequences represented by SEQ ID NOS:1 and 4 with the sequence represented by SEQ ID NO:7. JNK3 also refers to polypeptides that are at least 85% identical to the amino acid sequences listed above, and to the nucleic acids encoding those polypeptides. Examples of these sequences and methods of isolating them are found in Gupta et al., supra, 1996; Kyriakis et al., supra; Martin et al., Brain Res. Mol. Brain Res., 35:45-57, 1996; and Mohit et al., Neuron, 14:67-78, 1995.

[0018] A "control" cell is a cell that is generally the same, e.g. genotypically and phenotypically, as the cell to which it is being compared (e.g., the cells can be sister cells), but which is not exposed to a test compound.

[0019] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0020] Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **FIG. 1A** is a schematic representation of the nucleic acid sequence of GenBank Accession No. U34819 (SEQ ID NO:1).

[0022] **FIG. 1B** is a schematic representation of the amino acid sequence of GenBank Accession No. U34819 (SEQ ID NO:2).

[0023] **FIG. 1C** is a schematic representation of the nucleic acid sequence of SEQ ID NO:3.

[0024] **FIGS. 2A-B** is a schematic representation of the nucleic acid sequence of GenBank Accession No. U34820 (SEQ ID NO:4).

[0025] **FIG. 2C** is a schematic representation of the amino acid sequence of GenBank Accession No. U34820 (SEQ ID NO:5).

[0026] **FIG. 2D** is a schematic representation of the nucleic acid sequence of SEQ ID NO:6.

[0027] FIGS. 3A-B is a schematic representation of the nucleic acid sequence of GenBank Accession No. U07620 (SEQ ID NO:7).

[0028] FIG. 3C is a schematic representation of the amino acid sequence of GenBank Accession No. U07620 (SEQ ID NO:8).

[0029] FIG. 4A is schematic representation of the nucleic acid sequence of GenBank Accession No. L27128 (SEQ ID NO:9).

[0030] FIG. 4B is a schematic representation of the amino acid sequence of GenBank Accession No. L27128 (SEQ ID NO:10).

[0031] FIG. 5A is a schematic representation of the nucleic acid sequence of GenBank Accession No. L35236 (SEQ ID NO:11).

[0032] FIG. 5B is a schematic representation of the amino acid sequence of GenBank Accession No. L35236 (SEQ ID NO:12).

[0033] FIG. 6 is a diagram of the wild type JNK3 gene locus, targeting vector, and the mutated or disrupted JNK3 gene locus.

[0034] FIG. 7 is a bar graph showing the temporal responses of wild type and JNK3(-/-) mice to kainic acid (KA) injection.

[0035] FIG. 8 is a bar graph showing the temporal responses of wild type and JNK3(-/-) mice to pentetrazole (PTZ) injection.

[0036] FIG. 9A is a schematic representation of the nucleic acid sequence of murine c-Jun (GenBank Accession No. X12740; SEQ ID NO:13).

[0037] FIG. 9B is a schematic representation of the amino acid sequence of murine c-Jun (GenBank Accession No. X12740; SEQ ID NO:14).

[0038] FIGS. 10A-B is a schematic representation of the nucleic acid sequence of murine c-Fos (GenBank Accession No. V00727; SEQ ID NO:15).

[0039] FIG. 10C is a schematic representation of the amino acid sequence of murine c-Fos (GenBank Accession No. V00727; SEQ ID NO:16).

[0040] FIG. 11 is a bar graph showing the level of KA-induced AP-1 activity at various times after KA induction as reflected by luciferase activity in JNK3(-/-) mice crossed with transgenic AP-1 luciferase mice.

[0041] FIG. 12 is a bar graph showing the level of KA-induced AP-1 activity as reflected by the relative level of luciferase activity in hippocampus (HP) and cerebellum (CB) of JNK3(-/-) mice and wild type(+/+) mice.

[0042] FIG. 13 is a diagram of the proposed chain of molecular events caused by KA leading to neuronal apoptosis.

[0043] FIG. 14 is a diagram of the trisynaptic connection within the hippocampal formation.

DETAILED DESCRIPTION

[0044] JNK protein kinase phosphorylates c-Jun and subsequently increases AP-1 transcriptional activity in response

to a specific group of stress signals (Whitmarsh et al, supra; Yang et al., supra). The neural-specific expression of JNK3 may render neurons particularly susceptible to physiological stress. In the experiments described herein, a remarkable resistance to kainic acid (KA)-induced seizures and apoptosis has been observed in JNK3-deficient mice. The resistance to KA neurotoxicity may be due to the elimination of a specific stress-response pathway mediated by the JNK3 isoform of JNK protein kinase. First, the administration of KA caused the phosphorylation of the NH₂-terminal activation domain of c-Jun and markedly increased AP-1 transcriptional activity in wild-type, but not in JNK3-deficient mice. Second, there was prolonged expression of phosphorylated c-Jun within the most vulnerable area of the hippocampus, further indicating that JNK activity may lead to neuronal apoptosis.

[0045] The findings reported herein are consistent with the dependence of KA neurotoxicity on excitatory circuitry (Nadler et al., Brain Res. 195:47-56, 1980). Since JNK3 is widely expressed in the nervous system and its activity is increased by many different stress signals (Gupta et al., supra), JNK3 may be involved in stress-induced apoptosis caused by a wide range of environmental insults.

[0046] The identification of JNK3 as a critical mediator of KA-induced excitatory neurotoxicity has clinical implications. The amino acid sequence of mouse, rat and human JNK3 is highly conserved (Kyriakis et al., supra; Gupta et al., supra; Martin et al., supra; Mohit et al., Neuron. 14:67-78, 1995). Moreover, the expression of the human JNK3 gene is also restricted to the nervous system and neuroendocrine system, is widely expressed in many brain subregions (Gupta et al., supra; Mohit et al., supra). It is therefore likely that the human and rodent JNK3 protein kinases have related or identical physiological functions. Neurotoxicity of the excitatory amino acids has been implicated in many neurological disorders ranging from acute ischemia to chronic neurodegenerative diseases (Choi, Neuron, 1:623-634, 1988; Lipton et al., N. Engl. J. Med. 330:613-622, 1994; Rothman et al., Annu. Neurol. 19:105-111, 1986). Previous therapeutic strategies have been focused on the prevention of calcium influx through cell surface channels, such as the NMDA-type glutamate receptor. To date, these approaches have only met with mixed results (Lipton et al., supra). JNK3 is therefore a target for therapeutic interventions when excitatory neurotoxicity involves JNK3-mediated apoptosis.

[0047] In the experiments described infra, homologous recombination was used to generate JNK3-deficient mice, and their responses to noxious stimuli were examined. KA, a potent excitotoxic chemical, elicits limbic seizures and neuronal cell death. The neurotoxicity of KA derives from the direct stimulation of the glutamate receptor at postsynaptic sites, and an indirect increase in the release of excitatory amino acids from presynaptic sites. It is well-documented that systemic application of KA induces the expression of various cellular immediate early genes (cIEGs), including c-Jun and c-Fos. Thus, the application of KA triggers a stress-response pathway in the brain in vivo. The experiments detailed infra demonstrate that KA induces phosphorylation of c-Jun and an increase in AP-1 transcriptional activity in the brain of wild-type mice. However, these effects of KA are markedly suppressed in the brains of JNK3-deficient mice. Moreover, JNK3-deficient mice

exhibit a remarkable resistance to KA-induced seizures and apoptosis of hippocampal neurons. These normal mice treated with KA represent a useful model of human disorders of the nervous system involving excitotoxicity.

[0048] Based on these experimental results, JNK3 was found to be an exceptional target for limiting excitotoxic damage. In particular, JNK3 is a target in screening protocols including protocols to screen for molecules that regulate JNK3 gene expression, JNK3 binding to its substrates, and JNK3 activity, as described below. The molecules found in these screens that effectively decrease JNK3 expression or activity are candidate drugs to be used to treat disorders of the nervous system involving excitotoxicity, including seizure disorders such as epilepsy, cerebrovascular disorders including ischemia, metabolic imbalance (e.g., hypoglycemia), injury due to extreme heat or cold, trauma (e.g., irradiation, spinal cord injury, pressure, and ionic imbalance), dementias such as Alzheimer's disease, Parkinson's disease, and neurodegenerative disorders (e.g., Huntington disease), and motoneuron disease (including amyotrophic lateral sclerosis) (Thompson, *Science*, 267:1456-1462, 1995; Coyle et al., *Science*, 262:689-695, 1993).

Methods of Screening for Molecules that Inhibit JNK3 Activity

[0049] The following assays and screens can be used to identify compounds that are effective inhibitors of JNK3 activity. The assays and screens can be done by physical selection of molecules from libraries, and computer comparisons of digital models of compounds in molecular libraries and a digital model of the JNK3 active site. The inhibitors identified in the assays and screens may act by, but are not limited to, binding to JNK3 (e.g., from mouse or human), binding to intracellular proteins that bind to JNK3, compounds that interfere with the interaction between JNK3 and its substrates, compounds that modulate the activity of a JNK3 gene, or compounds that modulate the expression of a JNK3 gene or a JNK3 protein.

[0050] Assays can also be used to identify molecules that bind to JNK3 regulatory sequences (e.g., promoter sequences), thus modulating gene expression. See, e.g., Platt, *J. Biol. Chem.*, 269:28558-28562, 1994.

[0051] The compounds that can be screened by the methods described herein include, but are not limited to, peptides and other organic compounds (e.g., peptidomimetics) that bind to a JNK3 protein or inhibit its activity in any way. Such compounds may include, but are not limited to, peptides; for example, soluble peptides, including but not limited to members of random peptide libraries (see, e.g., Lam et al., *Nature* 354:82-84, 1991; Houghten et al., *Nature* 354:84-86, 1991), and combinatorial chemistry-derived molecular libraries made of D- and/or L-amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang et al., *Cell* 72:767-778, 1993), and small organic or inorganic molecules.

[0052] Compounds and molecules are screened to identify those that affect expression of a JNK3 gene or some other gene involved in regulating the expression of JNK3 (e.g., by interacting with the regulatory region or transcription factors of a gene). Compounds are also screened to identify those that affect the activity of such proteins (e.g., by inhibiting JNK3 activity) or the activity of a molecule involved in the regulation of JNK3.

[0053] Computer modeling or searching technologies are used to identify compounds, or identify modified compounds that modulate or are candidates to modulate the expression or activity of a JNK3 protein. For example, compounds likely to interact with the active site of the JNK3 protein are identified. The active site of JNK3 can be identified using methods known in the art including, for example, analysis of the amino acid sequence of a molecule, and from a study of complexes formed by JNK3 with a native ligand (e.g., ATF2 or c-Jun). Chemical or X-ray crystallographic methods can be used to identify the active site of JNK3 by the location of a bound ligand such as c-Jun or ATF2.

[0054] The three-dimensional structure of the active site can be determined. This can be done using known methods, including X-ray crystallography, which can be used to determine a complete molecular structure. Solid or liquid phase NMR can be used to determine certain intra-molecular distances. Other methods of structural analysis can be used to determine partial or complete geometrical structures. Geometric structure can be determined with a JNK3 protein bound to a natural (e.g., c-Jun or ATF2) or artificial ligand which may provide a more accurate active site structure determination.

[0055] Computer-based numerical modeling can be used to complete an incomplete or insufficiently accurate structure. Modeling methods that can be used are, for example, parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups are necessary, and can be selected from force fields known in physical chemistry. Information on incomplete or less accurate structures determined as above can be incorporated as constraints on the structures computed by these modeling methods.

[0056] Having determined the structure of the active site of a JNK3 protein, either experimentally, by modeling, or by a combination of methods, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. The compounds identified in such a search are those that have structures that match the active site structure, fit into the active site, or interact with groups defining the active site. The compounds identified by the search are potential JNK3 modulating compounds.

[0057] These methods may also be used to identify improved modulating compounds from an already known modulating compound or ligand. The structure of the known compound is modified and effects are determined using experimental and computer modeling methods as described herein. The altered structure is compared to the active site structure of a JNK3 protein to determine or predict how a particular modification to the ligand or modulating compound will affect its interaction with that protein. Systematic variations in composition, such as by varying side groups, can be evaluated to obtain modified modulating compounds or ligands of preferred specificity or activity.

[0058] Given the teachings herein, additional experimental and computer modeling methods useful to identify modu-

lating compounds based on identification of the active sites of a JNK3 protein and related transduction and transcription factors can be developed by those skilled in the art.

[0059] Examples of molecular modeling systems are the QUANTA programs, e.g., CHARMM, MCSS/HOOK, and X-LIGAND, (Molecular Simulations, Inc., San Diego, Calif.). QUANTA provides a modeling environment for two dimensional and three dimensional modeling, simulation, and analysis of macromolecules and small organics. Specifically, CHARMM analyzes energy minimization and molecular dynamics functions. MCSS/HOOK characterizes the ability of an active site to bind a ligand using energetics calculated via CHARMM. X-LIGAND fits ligand molecules to electron density patterns of protein-ligand complexes. The program also allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

[0060] Articles reviewing computer modeling of compounds interacting with specific proteins can provide additional guidance. For example, see, Rotivinen et al., *Acta Pharmaceutica Fennica* 97:159-166, 1988; Ripka, *New Scientist* 54-57 (Jun. 16, 1988); McKinaly and Rossmann, *Ann. Rev. Pharmacol. Toxicol* 29:111-122, 1989; Perry and Davies, *OSAR: Quantitative Structure-Activity. Relationships in Drug Design* pp. 189-193 (Alan R. Liss, Inc., 1989); Lewis and Dean, *Proc. R. Soc. Lond.* 236:125-140, 141-162, 1989; and, regarding a model receptor for nucleic acid components, see Askew et al., *Am. J. Chem. Soc.* 111:1082-1090. Computer programs designed to screen and depict chemicals are available from companies such as MSI (supra), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Gainesville, Fla.). These applications are largely designed for drugs specific to particular proteins; however, they may be adapted to the design of drugs specific to identified regions of DNA or RNA. Commercial sources of chemical libraries can be used as sources of candidate compounds. Such chemical libraries can be obtained from, for example, ArQule, Inc. (Medford, Mass.).

[0061] In addition to designing and generating compounds that alter binding, as described above, libraries of known compounds, including natural products, synthetic chemicals, and biologically active materials including peptides, can be screened for compounds that are inhibitors or activators.

[0062] Compounds identified by methods described above may be useful, for example, for elaborating the biological function of JNK3 gene products and in treatment of disorders in which JNK3 activity is deleterious. Assays for testing the effectiveness of compounds such as those described herein are further described below.

In Vitro Screening Assays for Compounds that Bind to JNK3 Proteins and Genes

[0063] In vitro systems can be used to identify compounds that can interact (e.g., bind) to JNK3 proteins or genes encoding those proteins. Such compounds may be useful, for example, for modulating the activity of JNK3 polypeptides or nucleic acids, elaborating their biochemistry, or treating disorders caused or exacerbated by JNK3 expression. These compounds may themselves disrupt normal function or can be used in screens for compounds that disrupt normal function.

[0064] Assays to identify compounds that bind to JNK3 proteins involve preparation of a reaction mixture of the

protein and the test compound under conditions sufficient to allow the two components to interact and bind, thus forming a complex that can be detected and/or isolated.

[0065] Screening assays for molecules that can bind to a JNK3 protein or nucleic acid can be performed using a number of methods. For example, a JNK3 protein, peptide, or fusion protein can be immobilized onto a solid phase, reacted with the test compound, and complexes detected by direct or indirect labeling of the test compound. Alternatively, the test compound can be immobilized, reacted with JNK3 polypeptide, and any complexes detected. Microtiter plates can be used as the solid phase and the immobilized component anchored by covalent or noncovalent interactions. Non-covalent attachment may be achieved by coating the solid phase with a solution containing the molecule, and drying. Alternatively, an antibody specific for JNK3 is used to anchor the molecule to the solid surface. Such surfaces may be prepared in advance of use, and stored. JNK3 antibodies anchored by covalent or noncovalent interactions. Non-covalent attachment may be achieved by coating the solid phase with a solution containing the molecule, and drying. Alternatively, an antibody specific for JNK3 is used to anchor the molecule to the solid surface. Such surfaces may be prepared in advance of use, and stored. JNK3 antibodies can be produced using conventional methods such as those described in Coligan et al. (*Current Protocols in Immunology*, John Wiley & Sons, Inc., 1994, see Volume 1, chapter 2).

[0066] In the assay, the non-immobilized component is added to the coated surface containing the immobilized component under conditions that permit interaction and binding between the two components. The unreacted components are then removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid phase. The detection of the complexes can be accomplished by a number of methods known to those in the art. For example, the nonimmobilized component of the assay may be prelabeled with a radioactive or enzymatic label and detected using appropriate means. If the non-immobilized entity was not prelabeled, an indirect method is used. For example, if the non-immobilized entity is a JNK3 polypeptide, an antibody against JNK3 is used to detect the bound molecule, and a secondary, labeled antibody is used to detect the entire complex.

[0067] Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected (e.g., using an immobilized antibody specific for a JNK3 protein).

[0068] Cell-based assays can be used to identify compounds that interact with JNK3 proteins. Cell lines that naturally express such proteins or have been genetically engineered to not contain the test compound indicates that the test compound is an inhibitor of JNK3 activity.

[0069] Inhibitors of JNK3 expression that act on the JNK3 promoter can be identified using a chimeric gene in which genomic sequences including the JNK3 promoter are fused to a reporter, for example firefly luciferase. Cultured cells (including neurons) transformed with this DNA are screened for the expression of luciferase activity. Compounds that inhibit luciferase activity in this high throughput assay can be confirmed by direct measurement of the endogenous JNK3 protein (by Western blotting) and JNK3 mRNA (by

Northern blotting) using methods known in the art (for example, see Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, 1994).

[0070] Candidate inhibitory compounds can be tested further in cell or tissue cultures as well as animal models. For example, cells expressing JNK3 are incubated with a test compound. Lysates are prepared from treated and untreated cells and Western blotted according to known methods. The blots are probed with antibodies specific for JNK3. A decrease in the amount of JNK3 expression in cultures treated with the test compound compared to untreated controls indicates that the test compound is a candidate for a drug to treat disorders associated with JNK3 expression.

Assays for Compounds that Interfere with JNK3/JNK3 Substrate Interactions

[0071] Molecules that disrupt the interaction between JNK3 and its substrates can be identified using assays that detect protein-protein interactions. For example, the yeast two-hybrid method detects protein interactions in vivo. However, an in vitro assay is preferable because candidate molecules may not be permeable to the yeast cell wall. An example of an in vitro assay for such test molecules that disrupt the interaction between JNK3 and a substrate includes the use of immobilized JNK3 or immobilized substrate (e.g., c-Jun) and incubation of the immobilized component with cell lysates or purified proteins in the presence and absence of a test molecule. In general, the test molecule is tested over a range of a 100 fold molar excess over the most abundant component (e.g., the component immobilized or in solution). If the test molecule is predicted to interact with the immobilized component of the assay, then it can be pre-incubated with that component before adding the cell lysate or purified protein. After washing away unbound material, the bound proteins are detected with antibodies (e.g., ELISA or Western blot) or through the use of labeled proteins (e.g. radioactive or fluorescent) using methods known in the art. Test molecules that decrease the amount of substrate bound to JNK3 are thus identified as molecules that interfere with JNK3/JNK3 substrate interactions.

Assays for Compounds that Ameliorate the Effects of JNK3 In Vivo

[0072] Compounds identified as above, or other candidate compounds that inhibit JNK3 activity in vitro may be useful for treating disorders involving JNK3 activity. These compounds can be tested in in vivo assays, for example, in animal models of disorders involving JNK3 activity. For example, transgenic mouse models of ALS have been described (Bruijn and Cleveland, *Neuropathol. Appl. Neurobiol.* 22:373-387, 1996; Dal Canto and Gurney, *Brain Res.* 676: 25-40, 1995; Cleveland et al., *Neurology* 47: Suppl 2, S54-61) as have transgenic models of Alzheimer's disease such as the PDAPP mouse and others (for examples, see Loring et al., *Neurobiol. Aging* 17:173-182, 1996). MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced dopaminergic neurotoxicity has been used as a model for Parkinson's disease in rodents and nonhuman primates (for example, Przedborski et al., *Proc. Nat'l. Acad. Sci. USA* 93:4565-4571, 1996).

[0073] Test compounds predicted to inhibit JNK3 activity are administered to animals, e.g., as described above, that

serve as models for the various disease paradigms. Treated animals are then assayed for inhibition of JNK3 activity. Such assays may be indirect or inferential, for example, improved health or survival of the animal indicates the efficacy of a test compound. Assays can also be direct, for example, a decrease in JNK3 or c-Jun expression can be measured by Northern analysis of neural tissue removed from an animal treated with a test compound. A decrease in the amount of JNK3 mRNA present in the sample from treated animals compared to untreated controls indicates that the test compound is inhibiting JNK3 expression. A decrease in the amount of c-Jun indicates that the test compound is inhibiting JNK3 expression or activity.

Antisense Constructs and Therapies

[0074] Treatment regimes based on an "antisense" approach involve the design of oligonucleotides (either DNA or RNA) that are complementary to JNK3 mRNAs. These oligonucleotides bind to the complementary mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, is a sequence sufficiently complementary to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may be tested, or triplex formation may assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0075] Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence, up to and including the AUG initiation codon, are generally most efficient for inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have also been shown to be effective for inhibiting translation (Wagner, *Nature*, 372:333, 1984). Thus, oligonucleotides complementary to either the 5' or 3' non-translated, non-coding regions of a JNK3 could be used in an antisense approach to inhibit translation of the endogenous human homolog of JNK3 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Examples of candidate antisense sequences for the 5' and 3' regions are; 5'-AAG AAA TGG AGG CTC ATA AAT ACC ACA GCT-3' (SEQ ID NO:17) and 5'-ATT GGA AGA AGA CCA AAG CAA GAG CAA CTA-3'(SEQ ID NO:18), respectively.

[0076] While antisense nucleotides complementary to the coding region of a JNK3 gene could be used, those complementary to transcribed untranslated regions are most preferred. Examples of this type of candidate sequence are 5'-TAA GTA AGT AGT GCT GTA TGA ATA CAG ACA-3' (SEQ ID NO:19) and 5'-TAC TGG CAA TAT ATT ACA GAT GGG TTT ATG-3'(SEQ ID NO:20).

[0077] Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation, but could be used in accordance with the invention. Whether designed to hybridize to the 5', 3', or coding region of a JNK3 mRNA, antisense nucleic acids should be at least

six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects, the oligonucleotide is at least 10 nucleotides, or at least 50 nucleotides in length.

[0078] Regardless of the choice of target sequence, in vitro studies are usually performed first to assess the ability of an antisense oligonucleotide to inhibit gene expression. In general, these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. In these studies levels of the target RNA or protein are usually compared with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide, and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

[0079] The oligonucleotides can be DNA or RNA, or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule or hybridization. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (as described, e.g., in Letsinger et al., Proc. Natl. Acad. Sci. USA 86:6553, 1989; Lemaitre et al., Proc. Natl. Acad. Sci. USA 84:648, 1987; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, for example, PCT Publication No. WO 89/10134), or hybridization-triggered cleavage agents (see, for example, Krol et al., BioTechniques 6:958, 1988), or intercalating agents (see, for example, Zon, Pharm. Res. 5:539, 1988). To this end, the oligonucleotide can be conjugated to another molecule, e.g., a peptide, hybridization-triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

[0080] The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethyl-aminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N-6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 2-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0081] The antisense oligonucleotide can also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0082] The antisense oligonucleotide can also include at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal, or an analog of any of these backbones.

[0083] The antisense oligonucleotide can include an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., Nucl. Acids. Res. 15:6625, 1987). The oligonucleotide is a 2'-O-methyl-ribonucleotide (Inoue et al., Nucl. Acids Res. 15:6131, 1987), or a chimeric RNA-DNA analog (Inoue et al., FEBS Lett. 215:327, 1987).

[0084] Antisense oligonucleotides of the invention can be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al. (Nucl. Acids Res. 16:3209, 1988), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. USA 85:7448, 1988).

[0085] The antisense molecules should be delivered to cells that express JNK3 proteins in vivo. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

[0086] However, it is often difficult to achieve intracellular concentrations of the antisense molecule sufficient to suppress translation of endogenous mRNAs. Therefore, an approach may be used in which a recombinant DNA construct comprises an antisense oligonucleotide placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in a patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous JNK3 transcripts and thereby prevent translation of that mRNA. For example, a vector can be introduced in vivo such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

[0087] Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Suitable promoters include, but are not limited to: the SV40 early promoter region (Bernoist et al., Nature 290:304, 1981); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al.,

Cell 22:787-797, 1988); the herpes thymidine kinase promoter (Wagner et al., Proc. Natl. Acad. Sci. USA 78:1441, 1981); and the regulatory sequences of the metallothionein gene (Brinster et al., Nature 296:39, 1988). Constructs may also be contained on an artificial chromosome (e.g., mammalian artificial chromosome; MAC; Harrington et al., Nature Genet. 15:345-355, 1997).

[0088] The production of a JNK3 antisense nucleic acid molecule by any gene therapeutic approach described above results in a cellular level of JNK3 protein that is less than the amount present in an untreated individual.

Ribozymes

[0089] Ribozyme molecules designed to catalytically cleave JNK3 mRNAs can also be used to prevent translation of these mRNAs and expression of JNK3 mRNAs (see, e.g., PCT Publication WO 90/11364; Saraver et al., Science 247:1222, 1990). While various ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy specific mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art (Haseloff et al., Nature 334:585, 1988). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the JNK3 mRNA, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[0090] Examples of potential ribozyme sites in human JNK3 include 5'-UG-3' sites which correspond to the initiator methionine codon at, for example, in human JNK3, about nucleotides 224-226, the codon for a downstream potential initiation site (nucleotides 338-340), and additional codons in the coding region, including nucleotides 698-670; 740-742; and 935-937.

[0091] The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes"), such as the one that occurs naturally in *Tetrahymena Thermophila* (known as the IVS or L-19 IVS RNA), and which has been extensively described by Cech and his collaborators (Zaug et al., Science 224:574, 1984; Zaug et al., Science, 231:470, 1986; Zug et al., Nature 324:429, 1986; PCT Application No. WO 88/04300; and Been et al., Cell 47:207, 1986). The Cech-type ribozymes have an eight base-pair sequence that hybridizes to a target RNA sequence, whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences present in JNK3 proteins.

[0092] As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, or targeting), and should be delivered to cells which express a JNK3 gene in vivo, e.g., the brain and spinal cord. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous JNK3 mRNAs and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0093] For any of the above approaches, the therapeutic JNK3 antisense or ribozyme nucleic acid molecule construct is preferably applied directly to the target area (e.g., the focal site of activity in a seizure disorder, the hippocampus in Alzheimer's disease, the *substantia nigra* in patients with Parkinson's disease), but can also be applied to tissue in the vicinity of the target area or even to a blood vessel supplying the target area.

[0094] For gene therapy, antisense, or ribozyme JNK3 expression is directed by any suitable promoter (e.g., the human cytomegalovirus, simian virus 40, or metallothionein promoters), and its production is regulated by any desired mammalian regulatory element. For example, if desired, enhancers that direct preferential gene expression in cells under excitotoxic induction can be used to direct antisense JNK3 expression in a patient with a seizure disorder.

[0095] JNK3 antisense or ribozyme therapy is also accomplished by direct administration of the antisense JNK3 or ribozyme RNA to a target area. This mRNA can be produced and isolated by any standard technique, but is most readily produced by in vitro transcription using an antisense JNK3 DNA under the control of a high efficiency promoter (e.g., the T7 promoter). Administration of antisense JNK3 RNA to target cells is carried out by any of the methods for direct administration of therapeutic compounds described herein.

Methods of Treating Disorders Involving JNK3 Expression or Activity

[0096] The invention also encompasses the treatment of disorders, especially in mammal, such as humans, in which JNK3 plays a damaging role. A number of disorders or the nervous system involving excitotoxicity, such as seizure disorders (e.g., epilepsy), dementias such as neurodegenerative disorders (e.g., Alzheimer's disease, Huntington disease), cerebrovascular disorders such as ischemia, motor neuron disease (including ALS), injuries caused by extreme heat or cold, trauma (e.g., irradiation, spinal cord injury, pressure, and ionic imbalance), metabolic imbalance (e.g., hypoglycemia) and Parkinson's disease, can be treated by the methods described herein. Without limiting the invention by committing to any particular theory, a substantial number of neurologic disorders are attributable, at least in part, to excitotoxicity which is mediated by the JNK3 pathway. Thus, inhibitors of this pathway, identified as described above, are useful for treatment of disorders involving excitotoxicity.

[0097] Therapy can be designed to reduce the level of endogenous JNK3 gene expression, e.g., using antisense or ribozyme approaches to inhibit or prevent translation of a JNK3 mRNA; triple helix approaches to inhibit transcription of the gene; or targeted homologous recombination to inactivate or "knock out" a gene or its endogenous promoter. The antisense, ribozyme, or DNA constructs described herein can be administered directly to the site containing the target cells; e.g., specific regions of the brain or the spinal cord. Antibodies or fragments of antibodies that recognize JNK3 or a JNK3 substrate, and that have been modified to be expressed or otherwise enter the cell can also be used therapeutically.

Effective Dose

[0098] Toxicity and therapeutic efficacy of the compounds of the invention, e.g., compounds that modulate JNK3

expression or activity, can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Polypeptides or other compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0099] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (that is, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

Formulations and Use

[0100] Pharmaceutical compositions for use in accordance with the present invention can be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

[0101] Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

[0102] For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (for example, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (for example, magnesium stearate, talc or silica); disintegrants (for example, potato starch or sodium starch glycolate); or wetting agents (for example, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (for example, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for

example, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

[0103] For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0104] The preferred methods of administering the compositions of the invention are by direct delivery of the compounds to the central nervous system, preferentially to the brain, especially near to or directly at the site of the disorder, e.g., the hippocampus in the case of Alzheimer's disease, the *substantia nigra* in the case of Parkinson's disease, and the focal site for seizure disorders. Accordingly, administration may be into a ventricle, intrathecal, or intracerebral ventricular. For example, an Omay reservoir-shunt with in-line filter can be surgically placed into the cisternal space. A therapeutic compound in an appropriate excipient (e.g., phosphate-buffered saline) is instilled into the shunt by injection on a prescribed basis.

[0105] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0106] The compounds can be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

[0107] The compounds can also be formulated in rectal compositions such as suppositories or retention enemas, for example, containing conventional suppository bases such as cocoa butter or other glycerides.

[0108] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0109] The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack

may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

[0110] The therapeutic compositions of the invention can also contain a carrier or excipient, many of which are known to skilled artisans. Excipients which can be used include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The nucleic acids, polypeptides, antibodies, or modulatory compounds of the invention can be administered by any standard route of administration. For example, administration can be parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, transmucosal, or oral. The modulatory compound can be formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection; gels or powders can be made for ingestion, inhalation, or topical application.

[0111] Methods for making such formulations are well known and can be found in, for example, "Remington's Pharmaceutical Sciences." It is expected that particularly useful routes of administration will be nasal or by direct infusion into the central nervous system.

EXAMPLES

Example 1

JNK3 Expression

[0112] A 351-bp sequence derived from the 5' region of the mouse JNK3 cDNA (nucleotides 62-412) was labelled with [³²P] by random priming and used as a probe to determine the tissue expression pattern of the JNK3 gene. Northern blot analysis was performed by standard methods on 2 mg samples of poly(A)⁺ mRNA isolated from testis, kidney, skeletal muscle, liver, lung, spleen, brain, and heart. All Northern blots were probed with [³²P]-labelled β-actin as a control to ensure loading of similar amounts of RNA in each lane. A strong signal corresponding to a 2.7 kb transcript, as well as a weak signal corresponding to a 7.0 kb transcript, were detected in brain. A weak signal corresponding to a 2.7 kb transcript was also detected in the heart. A signal corresponding to a 2.4 kb transcript was detected in the testis. JNK3 expression was not detected in the other tissues examined.

[0113] In situ hybridization analysis has indicated that JNK3 is expressed in many regions of the brain (Martin et al., supra). Total RNA (10 mg) was therefore isolated from different regions of mouse brain (cerebellum, cerebral cortex, hippocampus, midbrain, thalamus, and brainstem) using the TRIzol reagent (Gibco-BRL), and analyzed by Northern blot using the JNK3 probe described above. A signal corresponding to a 2.7 kb transcript was detected in all sections of the brain examined, and was most abundant in the hippocampus.

Example 2

Targeted Disruption of the JNK3 Gene

[0114] To generate JNK3-deficient mice, a targeting vector was designed to replace an internal 4 kb MscI-SpeI JNK3

genomic fragment with a PGKneo cassette. A map of the JNK3 gene, the JNK3 targeting vector, and the predicted structure of the mutated JNK3 gene are shown diagrammatically in **FIG. 6**. Restriction enzyme sites are indicated (B, BamHI; Hp, HpaI; M, MscI; Nco, NcoI; R, EcoRI; Spe, SpeI). A 10-kb NotI-EcoRI (the NotI site was vector derived) JNK3 fragment was cloned from a λ FixII phage library of a 129/Sv mouse strain (Stratagene Inc.). The targeting vector was constructed by inserting a 4.0 kb MscI fragment from the 5' end of the JNK3 genomic fragment, a 1.6 kb PGK-neo cassette (Negishi et al., Nature 376:435-438, 1995) and a 1.8-kb SpeI-NcoI fragment of the 3' end of the JNK3 fragment into pBluescript KS vector (Stratagene Inc.) using appropriate linkers. The targeting vector contains a 2.6-kb PGK tk cassette (Negishi et al., supra) flanking the 5' end of the JNK3 genomic sequence for negative-selection of mutant ES cells (Mansour et al., 336:348-352, 1988). The region replaced in the JNK3 gene by the targeting vector encompasses one and a half exons encoding amino acids 211 to 267 of JNK3 (as shown in **FIG. 5B**). This region includes the tripeptide dual phosphorylation motif Thr-Pro-Tyr (TPY) that is characteristic of the JNK group and required for protein kinase activity (Dèrijard et al., supra). The two hatched boxes shown in the JNK3 locus correspond to subdomains VIII and IX (encoding amino acid residues 189-267 in the JNK3 protein shown in **FIG. 5B**) of JNK3.

[0115] The targeting vector was linearized with NotI and electroporated into W9.5 embryonic stem (ES) cells. Genomic DNA from transfectants resistant to G418 (200 mg/ml)(Gibco BRL) and gancyclovir (2 mM) (Syntex, Palo Alto, Calif.) were isolated and screened by Southern blot analysis. Southern blot analysis of 104 independent G418- and gancyclovir-resistant clones revealed three clones containing the desired homologous recombination event (targeting frequency 2.9%). Chimeric mice were generated by injecting these ES cells into C57BL/6 (B6) mouse blastocysts.

[0116] Southern blots of EcoRI-restricted DNA derived from the tails of these chimeric mice were probed with the radiolabeled 351 bp JNK3 probe. EcoRI digestion resulted in a 12 kb band corresponding to the wild-type (endogenous allele), and a 4.2 kb band corresponding to the mutant (disrupted allele).

[0117] Two clones mediated germline transmission of the disrupted JNK3 allele into the next generation of mice. Heterozygotes (+/-) were intercrossed to generate homozygous mutant mice (-/-) that were identified by Southern blot analysis of genomic DNA. Total RNA isolated from mouse brain was examined by Northern blot analysis. The blot was probed with a random-primed ³²P-labeled mouse JNK3 cDNA probe, then stripped and sequentially reprobed with mouse JNK1 and β-actin cDNA probes. The major JNK3 transcript in brain is 2.7 kb, and the JNK1 transcripts in mouse brain are 2.3 and 4.4 kb. Blots hybridized with a JNK3 cDNA probe detected transcripts in wild-type (+/+), but not in homozygous knockout (-/-) mice.

[0118] Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was used to confirm that JNK3 transcripts were absent in the homozygous JNK3 (-/-) brain. A JNK1 probe (447 bp) was amplified from mouse brain RNA by RT-PCR (Yang et al., supra) using the amplimers 5'-GTGTGCAGCTTATGATGCTATTCTTGAA-3' (SEQ ID

NO:21) and 5'CGCGTCACCACATACGGAGTCATC-3' (SEQ ID NO:22). RT-PCR detection (Yang et al., supra) of JNK3 mRNA in mouse tissues was performed by RT-PCR using the amplimers: 5'-GTGGAGGAGTTCCAAGATGTCTACT-3' (SEQ ID NO: 23) and 5'-TGGAAAGAGCT-TGGGGAAGGTGAG-3' (SEQ ID NO:24) to yield specific 537 bp DNA product. RNA isolated from mouse brain was amplified with primers specific for HPRT as a control. These experiments confirmed the absence of JNK3 transcripts in the homozygous JNK3 (-/-) brain.

[0119] Protein kinase assays were performed to show that JNK3 (-/-) mouse brain was deficient in JNK3 activity. In these experiments, JNK3 kinase activity in brain lysates was measured after immunodeletion of JNK1 and JNK2 by in-gel protein kinase assays using the substrate GST-cJun (Dèrijard et al., supra) When mouse hippocampal lysates (30 μ g) from wild type (+/+) and homozygous knockout (-/-) brains were assayed, the 55 kD and 46 kD JNK3 isoforms were detected in wild type but not JNK3 (-/-) mice, confirming that JNK3 (-/-) mouse brain was deficient in JNK3 kinase activity. Together, these data demonstrate that the targeted disruption of the JNK3 gene resulted in a null allele.

[0120] The JNK3(-/-) mice were fertile and of normal size. Histological surveys of a variety of tissues revealed no apparent abnormality using hematoxylin and eosin (H & E) staining of heart, lung, thymus, spleen, lymph nodes, liver, kidney, and skeletal muscle. JNK3(-/-) and wild-type mouse brains were examined by immunocytochemical analysis of a pyramidal neuronal marker (MAP-2), interneuronal markers calbindin and parvalbumin, an astrocyte marker (glial fibrillary acidic protein; GFAP; Hsu et al., J. Histochem. Cytochem. 29:577-580, 1981), and Nissl's stain (Hsu et al., supra). These studies revealed that JNK3(-/-) mice had apparently normal development and structural organization of the brain. A comparable number of motor neurons were found in the facial nucleus in wild-type and JNK3(-/-) mice (2150-2300 neurons per nucleus at post-natal day 10, n=4). The neurons were identified by morphology and were counted by a double-blind assay of serial sections throughout the facial nuclei of wild-type and JNK3(-/-) mice. Thus, there is no apparent developmental abnormality, including cell death, in JNK3 (-/-) mice.

Example 3

JNK3 Deficient Mice are Resistant to KA-Induced Seizures

[0121] JNK3(-/-) mice and their wild-type littermates were injected intraperitoneally (i.p.) with 30 mg/kg KA to induce seizures (Ben-Ari, supra). In wild-type mice, the administration of KA first induced "staring spells" with abnormal body posture, then progressed to head nodding ("wet-dog shakes"), fore-paw tremor, rearing, loss of postural control, and eventually, continuous convulsions. The seizure activities typically subsided one hour after injection. Wild-type and heterozygous mice developed motor symptoms of seizures, including rearing, at 30 to 40 minutes post-injection. The JNK3 (-/-) mice, in contrast, developed much milder symptoms, mainly consisting of "staring" spells and occasional myoclonic tremors. At this dose, JNK3 (-/-) mice did not develop grand mal seizures and recovered much faster than did wild-type and heterozygous mice.

JNK3 (-/-) mice developed seizures of comparable severity to wild-type mice only at higher dosages of KA (45 mg/kg, i.p.). However, at this high dose of KA, more than 60% of wild-type mice died from continuous tonic clonic convulsions, while all of the JNK3(-/-) mice survived. These results indicate that JNK3(-/-) mice were resistant to the effect of the excitotoxin KA. Further, JNK3(-/-) mice recovered from the drug administration more rapidly than wild-type mice (FIG. 7). Seizure classifications as shown in FIGS. 7 and 8 are: 1, arrest of motion; 2, myoclonic jerks of the head and neck, with brief twitching movements; 3, unilateral clonic activity; 4, bilateral forelimb tonic and clonic activity; and 5, generalized tonic-clonic activity with loss of postural tone, often resulting in death.

Example 4

Resistance to Pentetrazole (PTZ)-Induced Seizures

[0122] Since the resistance to KA-induced seizures varied between littermates (+/+ and +/- are less resistant than -/- mice), the observed differential susceptibility cannot be attributed to a difference between mouse strains (Schauwecker and Steward, Proc. Nat. Acad. Sci. USA 94:4103-4108, 1997). However, the resistance of JNK3 deficient mice to KA-induced seizures could be due to decreased drug penetration across the blood-brain barrier or an increased GABA (gamma-aminobutyric acid) inhibitory postsynaptic potential (IPSP), or the ablation of a specific signal transduction pathway mediated by the JNK3 protein kinase. To distinguish between these possibilities, the response of JNK3 (-/-) and wild-type mice to another epileptogenic agent, pentetrazole (PTZ) (Sigma), was examined. PTZ was selected due to its ability to induce seizures by blocking the GABA-IPSPs (Ben-Ari et al., Neurosci. 6:1361-1391, 1981).

[0123] JNK3(-/-) mice and wild-type littermates developed seizures of comparable severity at all tested dosages of PTZ (30, 40, 50, 60 mg/kg, i.p.; FIG. 8). Moreover, unlike the slow progression of motor symptoms seen in the KA-induced seizures, PTZ induced abrupt general tonic-clonic seizures within five minutes after injection, presumably reflecting that its epileptogenic mechanism works solely through extracellularly inhibition of the GABA-IPSP. Thus, the differential susceptibility to KA toxicity in JNK3(-/-) mice can neither be explained as a consequence of poor drug delivery to the nervous system nor by potent GABA-IPSPs in the neural circuit. Furthermore, we examined the expression of the kainate-type glutamate receptor subunits GluR5-7 (Pharmingen cat. no. 60006E) by immunocytochemistry using standard methods.

[0124] Pyramidal neurons in the hippocampal CA1 subfield were most prominently labeled by the Glu5-7 antibody. Both wild-type and JNK3(-/-) mice showed prominently labeled apical dendrites arising from lightly labeled somata in the CA1 subfield of the hippocampus, a pattern similar to the primate hippocampus (Good et al., Brain Research 624:347-353, 1993). In addition to kainate-type subunit GluR5-7, the expression pattern of the GluR1 subunit that is essential to various glutamate receptor subtypes and the intracellular calcium-binding proteins parvalbumin and calbindin that may buffer the influx of extracellular calcium were also indistinguishable between JNK3(-/-) and wild-type mice. Together, these results indicate no apparent

structural abnormality that might be responsible for the resistance of JNK3(-/-) mice to KA-induced excitotoxicity.

Example 5

Attenuation of KA-Induced Phosphorylation of c-Jun

[0125] The systemic administration of KA in wild-type mice may induce a stress-response pathway mediated by the JNK3 protein kinase. To explore this possibility, the expression of the immediate-early genes c-fos and c-jun (Morgan et al., *Annu. Rev. Neurosci.* 14:421-451, 1991; Smeyne et al., *Nature* 363:166-169, 1993; Kasof et al., *J. Neurosci.* 15:4238-4249, 1995) was examined to determine whether KA imposed an equivalent level of noxious stimulation on wild-type and JNK3(-/-) mice. Total RNA was extracted from the hippocampi of mice sacrificed before and at 0.5, 2, 4, or 8 hours after KA injection (30 mg/kg, i.p.), and Northern blots were probed with murine c-fos and c-jun probes. The c-jun probe was a 207 bp fragment corresponding to nucleotides 888-1094 (FIG. 9) of the murine c-Jun cDNA. The c-Fos probe was a 347 bp fragment of the murine c-Fos gene (exon 4; base pairs 2593-2939) (FIG. 10). Both JNK3(-/-) and wild type mice exhibited a comparable level of rapid induction of c-fos and c-jun transcripts, which gradually declined four hours after injection.

[0126] To further define this phenomenon, the distribution of KA-induced c-Fos and c-jun immunoreactivity along the synaptic circuit of the hippocampus was examined. In these experiments, homozygous mutant and control wild-type mice were killed and fixed by transcardial perfusion of 4% paraformaldehyde at 2 or 6 hours after the injection of KA (30 mg/kg, i.p.). Brains from both groups were removed, post-fixed for one hour, and sectioned on a Vibratome (40 mm thick). Tissue sections were processed by immunocytochemistry to detect the expression of c-Jun (Santa Cruz, cat# sc-45), c-Fos (Santa Cruz, cat# sc-52), and phospho-specific c-Jun (Ser-73) (New England Biolabs, #9164S). Sections were floated in a solution of the primary antibody (diluted 200x in PBS) and incubated overnight at room temperature. Secondary antibody incubation, avidin-biotin conjugated peroxidase (Vectastain Elite ABC kit, Vector Lab.), and DAB (3,3'-diaminobenzidine, Sigma) reactions were performed using standard procedures (Hsu et al., supra). In the absence of KA, there was no detectable c-Fos expression and only a few c-Jun-positive cells within the dentate gyrus. Two hours after KA injection (30 mg/kg, i.p.), there was a large increase in c-Fos immunoreactivity throughout the hippocampal region that was the same in both wild-type and JNK3(-/-) mice. Simultaneously, there was an increase in the number of c-Jun-positive cells in the dentate gyrus and the CA3 region of the hippocampus in both wild type and JNK3(-/-) mice. By six hours after KA injection, the expression of c-Jun extended to the CA1 region in both wild-type and JNK3(-/-) mice. The induction of c-Fos and C-Jun is generally accepted as an indicator of neuronal activity following noxious stimulation (Morgan et al., supra). The comparable induction level, time-course, and distribution of c-Jun and c-Fos-labeled cells suggests that JNK3(-/-) and wild-type mice were subject to an equivalent level of noxious stress by systemic administration of KA.

[0127] C-Jun is activated by phosphorylation of the NH₂-terminal activation domain by JNK. The expression of

phosphorylated c-jun provides another measure of whether JNK-like activity was present in JNK3(-/-) mice. The expression of phosphorylated c-Jun was examined using an antibody raised against c-jun phosphorylated at Ser-73, one of the sites phosphorylated by JNK (Whitmarsh et al., supra; Dèrijard et al., supra; Kyriakis et al., supra). Prior to challenge with KA, no cells were labeled by the antibody in either wild type or JNK3(-/-) mice. By two hours after KA injection, there was a high level of phosphorylated c-Jun in the dentate gyrus and the CA3/CA4 region of the hippocampus in wild-type mice. In contrast, only a trace amount of phosphorylated c-Jun was detected in the JNK3(-/-) mice. Thus, there was either a decreased level or less sustained phosphorylation of c-Jun in the JNK3(-/-) mice.

[0128] In addition, there was a dynamic change of the distribution of phosphorylated c-Jun in the wild type mouse hippocampus. By six hours after KA injection, the expression of phosphorylated c-Jun subsided in the dentate gyrus and progressed to a restricted area in the hippocampal CA3 region. Under higher magnification, it was apparent that the expression of phosphorylated c-Jun surrounded a focus of cell destruction. In contrast, no labeling of phosphorylated c-Jun was detected in the JNK3(-/-) mice at the same time point. The hippocampal CA3 region is well documented as the most vulnerable structure to the KA excitotoxicity, presumably due to both a high KA binding affinity (Berger et al., supra) and a potent excitatory synaptic connection between CA3 pyramidal neurons (Westbrook et al., *Brain Research* 273:97-109, 1983). These results indicate that JNK3 is required for the phosphorylation of c-Jun induced by KA.

Example 6

Attenuation of KA-Induced AP-1 Transcriptional Activity

[0129] Since the phosphorylation of c-Jun is an important initial event during the induction of AP-1 transcriptional activity (Whitmarsh et al., supra; Yang et al., supra), whether the observed attenuation of c-Jun phosphorylation would lead to decreased induction of AP-1 transcriptional activity in JNK3(-/-) mice was examined. JNK(-/-) mice were crossed with transgenic AP-1 luciferase. (AP1-luc) mice (Rincon et al., *Embo. J.* 13:4370-4381, 1994) and progeny back crossed. The JNK3(-/-)/API-Luc(-/+) mice were used in experiments with JNK3(+/+) mice to compare the level of KA-induced AP-1 transcriptional activity in the presence or absence of JNK3. The AP1-luc mice contain the firefly luciferase gene under the control of four copies of a consensus AP-1 binding site in the context of the minimal rat prolactin promoter. It has been established that the expression of luciferase in these mice is due to the presence of the AP-1 regulatory element.

[0130] In the luciferase assay, mice were sacrificed at intervals after injection of KA (30 mg/kg, i.p.), and relative luciferase activity compared with that detected in hippocampal lysates obtained from mice injected with vehicle (saline). Mice were decapitated, brains were dissected, and brain tissues were immediately lysed in buffer containing 25 mM Hepes pH 7.4, 1% TRITON®X-100, 1 mM EDTA, 1 mM phenylmethyl sulfonyl fluoride, and 10 µg/ml leupeptin (Promega, Madison, Wis.). Luciferase activity was measured as described in Rincon and Flavell (*Embo. J.* 13:4370-

4381, 1994). The injection of KA (30 mg/kg, i.p.) caused a large induction of AP-1 transcriptional activity in the hippocampus of wild-type mice, as evidenced by the induction of luciferase activity. Luciferase activity in wild type mice was detectable by six hours, gradually increased to the peak at three days, and persisted for at least seven days (**FIG. 11**). Control experiments demonstrated that the injection of vehicle (saline) did not cause induction of luciferase activity in the AP1-luc mice.

[0131] The relative luciferase activity in the hippocampus and cerebellum prepared from wild-type (+/+) and JNK3 (-/-) mice was measured following KA injection. The results are shown in **FIG. 12**. Each time point represents the mean of three to five (+SEM) individual animals. The induction of luciferase activity was most prominent in the hippocampus, where a markedly greater induction of phosphorylation of c-Jun was observed, as compared to the cerebellum and the cerebral cortex. The induction of AP-1 activity was significantly reduced in the JNK3 (-/-) mice with the AP1-luc transgene as compared to the wild type mice. At 15 hours after injection of KA, there was approximately four-fold greater AP-1 activity in the hippocampus of wild-type mice compared with JNK3(-/-) mice. At three days after injection, the AP-1 activity was more than six times higher in the hippocampus of wild-type compared with JNK3(-/-) mice. Together, these data demonstrate that the disruption of the JNK3 gene suppressed KA-induced phosphorylation of c-Jun and AP-1 transcription activity in the hippocampus in vivo.

Example 7

Resistance to KA-Induced Apoptosis

[0132] One unique feature of KA among other epileptogenic agents is its potency in inducing neuronal cell death (Ben-Ari, supra; Schwob et al., supra). Since this property of cell destruction is paralleled by a sustained level of AP-1 transcriptional activity, it has been suggested that AP-1 mediates KA-induced-neuronal death (Kasof et al., supra; Schwarzschild et al., J. of Neurosci. 17:3455-3466, 1997). Wild-type and JNK3(-/-) mouse brains were therefore examined after treatment with KA to determine whether the attenuation of AP-1 transcriptional activity in JNK3 (-/-) mice altered the extent of neuronal damage (Ben-Ari, supra; Ben-Ari et al., supra; Schwob et al., Neurosci. 5:991-1014, 1980).

[0133] These experiments were performed as follows. Wild-type and JNK3 (-/-) mice were killed and fixed by transcardial perfusion of 4% paraformaldehyde and 1.5% glutaraldehyde three days after the injection of KA (30 mg/kg, i.p.). Semithin and thin sections of brain were prepared using a Vibratome and embedded in Epon. Tissue blocks were prepared using a microtome with a diamond tube for 1 μ m-thick semithin sections examined by toluidine blue staining, and for ultrathin sections examined by electron microscopy. Nissl's stain was used for initial examination of damage to the hippocampus (Kluver et al., J. Neuropath. Exp. Neuro. 12:400-403, 1953). GFAP immunocytochemistry was also used to assess cell destruction in the hippocampus (Hsu et al., supra). Nissl's staining was also performed as described above. TUNEL assays, used to evaluate apoptosis, were performed using cryostat sections (50 μ m) of cerebral hemispheres that were cryo-

protected with sucrose. The TUNEL assay was modified from the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling assay (Gavrieli et al., J. Cell. Biol. 119:493-501, 1992). Briefly, tissue sections, directly mounted on a salinated slide, were permeabilized with 2% TRITON® X-100 (20 minutes at room temperature) and then incubated for nick end-labeling for 2 hours at 37° C. using 0.32 U/ μ l TdT (Boehringer Mannheim, cat# 220582) and 2 μ M digoxigenin-11-dUTP (Boehringer Mannheim, cat# 1573152) in a final volume of 40 μ l. The tissues were incubated with anti-digoxigenin antibody (Boehringer Mannheim, cat# 1333062) diluted 500-fold, and processed for immunocytochemistry using standard procedures (Hsu et al., supra).

[0134] The damage to the hippocampus caused by KA was initially examined by Nissl's stain. The KA-induced cell loss caused either a breach of staining of the pyramidal neurons in the CA3 region or a diffuse sparse staining throughout the CA1 subfield. To corroborate the cell destruction revealed by Nissl's stain, the TUNEL method was applied to detect apoptosis. Groups of small pyknotic nuclei and positively TUNEL-labeled cells were found in the hippocampal CA3 subfield devoid of Nissl's staining. Similarly, a high percentage of pyramidal neurons showing pyknotic nuclei and shattered apical dendrites and numerous strongly TUNEL-labeled cells were located in the hippocampal CA1 subfield which exhibited decreased Nissl's staining. Since the TUNEL method and the pyknosis morphology only indicated the extent of cell damage at one time point of a dynamic process, immunostaining of damage-induced GFAP was also used as an independent assessment of the extent of cell destruction in the hippocampus. Consistent with the patterns of Nissl's, toluidine, and TUNEL staining, an increased number of strongly GFAP-labeled astrocytes was found either in the hippocampal CA3 or CA1 regions. Thus, a combination of Nissl's stain, GFAP immunocytochemistry, TUNEL method, and toluidine stain of semithin sections was used to classify the KA-induced damage in the mouse hippocampus.

[0135] A total of 17 wild-type and 18 JNK3(-/-) mice were examined. Results are shown in Table 1 (below). The table was compiled from two sets of data. First, wild-type (n=11) and JNK3 (-/-) (n=10) mice were sacrificed on the fifth day after a single injection of KA (30 mg/kg, i.p.). Second, wild-type (n=6) and JNK3 (-/-) (n=8) mice received an injection of KA (30 mg/kg, i.p.) for five consecutive days and examined two days following the final injection. The severity of the hippocampal damage in wild-type mice was comparable in experiments using both protocols. The ratio of no cell loss/CA3 lesion/CA3+CA1 lesion was 2/7/2 in the single-injection experiments, and 2/2/2 in the multiple injection experiments.

TABLE 1

Kainate-induced neuronal damage (number of animals)		
JNK3 genotype	+/+	-/-
No perceivable cell loss	4	18
Selective CA3 cell loss	9	0
Including CA1 cell loss	4	0

[0136] The hippocampal CA3 region was the most susceptible to KA-induced damage in the wild-type mice (9/17;

53%). Cell loss was indicated by decreased crystal violet staining in the CA3 region. Using the TUNEL method (Gavrieli et al., supra), which identifies DNA fragmentation in the dying cells, groups of labeled cells were found in the damaged region. A cluster of pyknotic nuclei was found in the CA3 region in toluidine-stained semithin sections. As a result of KA-induced damage, there was selective glial proliferation confined to the CA3 region, as indicated by the strong immunostaining of GFAP. In some wild-type animals, massive cell loss was observed throughout the entire hippocampal CA1 region (4/17; 24%). Similarly, damage to the CA1 region was revealed by decreased crystal violet staining, positively TUNEL-labeled cells, pyknotic nuclei, shattered apical dendrites of pyramidal neurons, and both hypertrophy and proliferation of GFAP-positive astrocytes.

[0137] In contrast, there was no apparent hippocampal damage in any of the JNK3(-/-) mice examined (n=18). The pattern of the Nissl's stain, TUNEL assay, toluidine blue staining of semithin sections, and GFAP immunostaining of the hippocampal region in the JNK3(-/-) mice was indistinguishable from that of untreated wild-type mice. Moreover, although JNK2(-/-) mice developed seizures of comparable severity at the sublethal dose of 45 mg/kg KA (a dose that is lethal for more than 60% of wild-type mice due to continuous convulsions), cell damage was nevertheless found in a much smaller percentage of animals (2/15, 13%; $p < 0.005$ by chi-square analysis, d.f.=1).

[0138] Methods of assessing apoptosis (e.g., TUNEL assay) can be used to evaluate whether JNK3 modulator is affecting apoptosis.

Example 8

Electron Microscopic Analysis of Ultrastructural Changes Associated with KA-Induced Neuronal Damage

[0139] Cortical neurons in vitro undergo either apoptosis or necrosis depending on the extracellular concentration of the glutamate analog N-methyl-D-aspartate-(NMDA) (Bonfoco et al., Proc. Natl. Acad. Sci. USA 92:7162-7166, 1995). The distinction between apoptosis versus necrosis in KA-induced neuronal damage is critical since necrosis is generally thought to represent a consequence of acute mechanical insult that is incompatible with an active cell death program involving de novo protein synthesis. The TUNEL results (supra) indicate the involvement of apoptosis. To further examine whether the neuronal death in vivo due to KA induction was apoptotic or necrotic, electron microscopy was employed to investigate the ultrastructural changes in the degenerated hippocampal neurons. The microscopic analysis suggested a series of morphological changes indicating neuronal damage in the wild-type mouse as a consequence of apoptosis. The initial event after KA injection (30 mg/kg i.p.) appeared to be compaction and segregation of chromatin in pyramidal neurons into electron-dense masses that abutted on the inner surface of the nuclear envelope. In contrast, the nuclei of the hippocampal neurons in the JNK3(-/-) mice following KA injection contained homogeneous electron-lucent euchromatin. At later stages, in wild type mice, there was convolution of the nuclear outline and condensation of the cytoplasm. The double-layered structure of the nuclear envelope in wild type mice remained largely intact in all of these morphological stages. Eventually the

degenerated neurons disintegrated resulting in numerous membrane-bounded apoptotic bodies. These morphological features are all consistent with the hallmarks of apoptosis (Kerr et al., Br. J. Cancer 26:239-257, 1972). Thus, it appeared that KA triggered a genetic program within the damaged neuron leading to apoptosis, which was abrogated in JNK3-deficient neurons.

[0140] These results suggest that KA-induced phosphorylation of the NH₂-terminal activation domain of c-Jun leads to increased AP-1 transcriptional activity and neuronal apoptosis. Without limitation to a particular theory, a proposed chain of molecular events caused by KA that lead to neuronal apoptosis is shown in FIG. 13.

[0141] Although systemic administration of KA causes cell damage predominantly localized in the hippocampal CA3 area, the significance of JNK3 in stress-induced neuronal apoptosis is not only restricted to this region. Several lines of evidence indicate that the particular vulnerability of the CA3 hippocampal neurons to KA is due to their unique cellular and synaptic properties. First, the hippocampal CA3 and CA4 regions have the highest density of KA-receptors (Berger et al., Neurosci. Lett. 39:237-242, 1983). Second, the recurrent synaptic excitation is particularly potent in the hippocampal CA3 region (Miles et al., J. Physiol. (London) 373:397-418, 1986). The recurrent excitation of the CA3 pyramidal neurons may sustain JNK3 signaling and therefore rapidly induce KA excitotoxicity. The observed progression of c-Jun phosphorylation from the dentate gyrus to the CA3 region is reminiscent of the synaptic circuitry of the hippocampus. A diagram of the trisynaptic connection within the hippocampal formation is shown in FIG. 14. The first synaptic relay (1) is from the afferent perforant path (pp) onto the granule cell of the dentate gyrus (DG). The second relay (2) follows the mossy fiber (mf) from the dentate gyrus to the CA3 hippocampal neurons. The third relay (3) is from the hippocampal CA3 to the CA1 region along the Schaffer collaterals (Sch). There are recurrent synaptic interactions of pyramidal neurons in the CA3 region.

Example 9

Assays for Detection of Inhibitors of JNK3 Protein Kinase Activity

[0142] Inhibitors of JNK3 can be identified in protein kinase assays. These assays can be performed using JNK3 purified from tissue (e.g., brain) or with recombinant enzyme. The recombinant JNK3 can be isolated from bacteria, yeast, insect, or mammalian cells using standard procedures. Assays of endogenous (natural) JNK3 are known in the art and assays of recombinant JNK3 have been described previously (Gupta et al., EMBO J. 15:2760-2770, 1996).

[0143] The protein kinase activity of JNK3 can be measured using ATP and protein substrates for JNK3 in an in vitro assay. These substrates include, but are not limited to, the transcription factors ATF2 and Elk-1 (Gupta et al., 1996, supra). The incorporation of phosphate into the substrate can be measured by several methods. One example is to measure the incorporation of radioactive phosphate (e.g., ³²P) into the substrate. The incorporation into the substrate can be measured following removal of unincorporated radioactivity by precipitation with trichloroacetic acid and recovery on

phosphocellulose paper or by polyacrylamide gel electrophoresis. The radioactivity can be monitored by scintillation counting, phosphorimager analysis, or by autoradiography. In general, methods for automated high throughput screens would not use radioactive materials. For this purpose a method is used to detect the phosphorylated substrate without a radioactive probe. In one approach the electrophoretic mobility of the substrate is examined. For example, ATF2 demonstrates a marked reduction in electrophoretic mobility following phosphorylation by JNK on Thr-69 and Thr-71 (Gupta et al., Science 267:389-393, 1995).

[0144] A second approach is to detect the phosphorylation of the substrate using immunochemical methods (e.g. ELISA). Antibodies that bind specifically to the phosphorylated substrates are prepared (monoclonal and polyclonal) and are commercially available (e.g., New England Biolabs, Promega Corp., and Upstate Biotechnology Inc.). The extent of substrate phosphorylation is then measured by standard ELISA assay using secondary antibodies coupled molecules suitable for to spectrophotometric or fluorometric detection using methods known in the art.

[0145] Molecules that inhibit JNK3 can be identified in a high throughput screen. A molecule that is a preferred candidate to treat excitotoxic disorders inhibits JNK3, but not other protein kinases, including related MAP kinases. Candidate molecules once identified can be optimized using combinatorial chemical methods or by the synthesis of related molecules. These molecules represent candidate drugs that can be tested for JNK3 therapy.

Example 10

Assays for Detection of Inhibitors of JNK3 Activation

[0146] The JNK protein kinases are activated by dual phosphorylation on Thr and Tyr within protein kinase sub-

domain VIII (Davis, Trends Biochem. Sci. 19:470-473, 1994). These sites of activating phosphorylation are conserved in JNK3 (Gupta et al., 1996, supra). Molecules that inhibit the activation of JNK3 by interfering with the phosphorylation of JNK3 can be identified by measurement of JNK3 activation in the presence and absence of candidate molecules. Cells expressing JNK3, e.g., neuronal cells, neuroendocrine cells, or cells that are engineered to express recombinant JNK3 (Gupta et al., 1996 supra), are exposed to environmental stress (e.g., depolarization, excitotoxic agents, UV radiation, heat, and anoxia) to activate JNK3. The state of JNK3 activation can be assessed by several methods. For example, JNK3 can be isolated, washed free of the candidate inhibitor, and the activation state of JNK3 monitored by protein kinase assay (supra). Alternatively, the activation of JNK3 can be probed using immunological methods using antibodies that bind to the Thr and Tyr phosphorylated (activated) form of JNK3. Antibodies that bind specifically to the Thr and Tyr phosphorylated enzyme can be prepared (monoclonal and polyclonal) and are commercially available (e.g., from New England Biolabs and Promega Corp.). The extent of substrate phosphorylation can then be measured by a standard ELISA assay using secondary antibodies coupled to spectrophotometric or fluorometric detection.

Other Embodiments

[0147] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 24

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1505 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 68...1459

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

TTATGCAAGA AACTGTTGAA TTAGACCCGT TTCCTATAGA TGAGAAACCA TACAAGCTGT	60
GGTATTT ATG AGC CTC CAT TTC TTA TAC TAC TGC AGT GAA CCA ACA TTG	109
Met Ser Leu His Phe Leu Tyr Tyr Cys Ser Glu Pro Thr Leu	

-continued

1			5			10												
GAT	GTG	AAA	ATT	GCC	TTT	TGT	CAG	GGA	TTC	GAT	AAA	CAA	GTG	GAT	GTG			157
Asp	Val	Lys	Ile	Ala	Phe	Cys	Gln	Gly	Phe	Asp	Lys	Gln	Val	Asp	Val			
15					20					25					30			
TCA	TAT	ATT	GCC	AAA	CAT	TAC	AAC	ATG	AGC	AAA	AGC	AAA	GTT	GAC	AAC			205
Ser	Tyr	Ile	Ala	Lys	His	Tyr	Asn	Met	Ser	Lys	Ser	Lys	Val	Asp	Asn			
				35					40					45				
CAG	TTC	TAC	AGT	GTG	GAA	GTG	GGA	GAC	TCA	ACC	TTC	ACA	GTT	CTC	AAG			253
Gln	Phe	Tyr	Ser	Val	Glu	Val	Gly	Asp	Ser	Thr	Phe	Thr	Val	Leu	Lys			
			50					55					60					
CGC	TAC	CAG	AAT	CTA	AAG	CCT	ATT	GGC	TCT	GGG	GCT	CAG	GGC	ATA	GTT			301
Arg	Tyr	Gln	Asn	Leu	Lys	Pro	Ile	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val			
		65					70					75						
TGT	GCC	GCG	TAT	GAT	GCT	GTC	CTT	GAC	AGA	AAT	GTG	GCC	ATT	AAG	AAG			349
Cys	Ala	Ala	Tyr	Asp	Ala	Val	Leu	Asp	Arg	Asn	Val	Ala	Ile	Lys	Lys			
	80					85					90							
CTC	AGC	AGA	CCC	TTT	CAG	AAC	CAA	ACA	CAT	GCC	AAG	AGA	GCG	TAC	CGG			397
Leu	Ser	Arg	Pro	Phe	Gln	Asn	Gln	Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg			
	95				100					105					110			
GAG	CTG	GTC	CTC	ATG	AAG	TGT	GTG	AAC	CAT	AAA	AAC	ATT	ATT	AGT	TTA			445
Glu	Leu	Val	Leu	Met	Lys	Cys	Val	Asn	His	Lys	Asn	Ile	Ile	Ser	Leu			
				115					120					125				
TTA	AAT	GTC	TTC	ACA	CCC	CAG	AAA	ACG	CTG	GAG	GAG	TTC	CAA	GAT	GTT			493
Leu	Asn	Val	Phe	Thr	Pro	Gln	Lys	Thr	Leu	Glu	Glu	Phe	Gln	Asp	Val			
			130					135					140					
TAC	TTA	GTA	ATG	GAA	CTG	ATG	GAT	GCC	AAC	TTA	TGT	CAA	GTG	ATT	CAG			541
Tyr	Leu	Val	Met	Glu	Leu	Met	Asp	Ala	Asn	Leu	Cys	Gln	Val	Ile	Gln			
		145					150					155						
ATG	GAA	TTA	GAC	CAT	GAG	CGA	ATG	TCT	TAC	CTG	CTG	TAC	CAA	ATG	TTG			589
Met	Glu	Leu	Asp	His	Glu	Arg	Met	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu			
	160					165						170						
TGT	GGC	ATT	AAG	CAC	CTC	CAT	TCT	GCT	GGA	ATT	ATT	CAC	AGG	GAT	TTA			637
Cys	Gly	Ile	Lys	His	Leu	His	Ser	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu			
	175				180					185					190			
AAA	CCA	AGT	AAC	ATT	GTA	GTC	AAG	TCT	GAT	TGC	ACA	TTG	AAA	ATC	CTG			685
Lys	Pro	Ser	Asn	Ile	Val	Val	Lys	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu			
			195						200				205					
GAC	TTT	GGA	CTG	GCC	AGG	ACA	GCA	GGC	ACA	AGC	TTC	ATG	ATG	ACT	CCA			733
Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala	Gly	Thr	Ser	Phe	Met	Met	Thr	Pro			
			210					215					220					
TAT	GTG	GTG	ACA	CGT	TAT	TAC	AGA	GCC	CCT	GAG	GTC	ATC	CTG	GGG	ATG			781
Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met			
		225					230					235						
GGC	TAC	AAG	GAG	AAC	GTG	GAT	ATA	TGG	TCT	GTG	GGA	TGC	ATT	ATG	GGA			829
Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile	Trp	Ser	Val	Gly	Cys	Ile	Met	Gly			
	240					245					250							
GAA	ATG	GTT	CGC	CAC	AAA	ATC	CTC	TTT	CCA	GGA	AGG	GAC	TAT	ATT	GAC			877
Glu	Met	Val	Arg	His	Lys	Ile	Leu	Phe	Pro	Gly	Arg	Asp	Tyr	Ile	Asp			
	255				260					265					270			
CAG	TGG	AAT	AAG	GTA	ATT	GAA	CAA	CTA	GGA	ACA	CCA	TGT	CCA	GAA	TTC			925
Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln	Leu	Gly	Thr	Pro	Cys	Pro	Glu	Phe			
				275					280					285				
ATG	AAG	AAA	TTG	CAA	CCC	ACA	GTA	AGA	AAC	TAT	GTG	GAG	AAT	CGG	CCC			973
Met	Lys	Lys	Leu	Gln	Pro	Thr	Val	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro			
			290					295					300					
AAG	TAT	GCG	GGA	CTC	ACC	TTC	CCC	AAA	CTC	TTC	CCA	GAT	TCC	CTC	TTC			1021
Lys	Tyr	Ala	Gly	Leu	Thr	Phe	Pro	Lys	Leu	Phe	Pro	Asp	Ser	Leu	Phe			

-continued																	
305					310					315							
CCA	GCG	GAC	TCC	GAG	CAC	AAT	AAA	CTC	AAA	GCC	AGC	CAA	GCC	AGG	GAC	1069	
Pro	Ala	Asp	Ser	Glu	His	Asn	Lys	Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp		
320					325					330							
TTG	TTG	TCA	AAG	ATG	CTA	GTG	ATT	GAC	CCA	GCA	AAA	AGA	ATA	TCA	GTG	1117	
Leu	Leu	Ser	Lys	Met	Leu	Val	Ile	Asp	Pro	Ala	Lys	Arg	Ile	Ser	Val		
335					340					345					350		
GAC	GAC	GCC	TTA	CAG	CAT	CCC	TAC	ATC	AAC	GTC	TGG	TAT	GAC	CCA	GCC	1165	
Asp	Asp	Ala	Leu	Gln	His	Pro	Tyr	Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ala		
355					360					365							
GAA	GTG	GAG	GCG	CCT	CCA	CCT	CAG	ATA	TAT	GAC	AAG	CAG	TTG	GAT	GAA	1213	
Glu	Val	Glu	Ala	Pro	Pro	Pro	Gln	Ile	Tyr	Asp	Lys	Gln	Leu	Asp	Glu		
370					375					380							
AGA	GAA	CAC	ACA	ATT	GAA	GAA	TGG	AAA	GAA	CTT	ATC	TAC	AAG	GAA	GTA	1261	
Arg	Glu	His	Thr	Ile	Glu	Glu	Trp	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val		
385					390					395							
ATG	AAT	TCA	GAA	GAA	AAG	ACT	AAA	AAT	GGT	GTA	GTA	AAA	GGA	CAG	CCT	1309	
Met	Asn	Ser	Glu	Glu	Lys	Thr	Lys	Asn	Gly	Val	Val	Lys	Gly	Gln	Pro		
400					405					410							
TCT	CCT	TCA	GGT	GCA	GCA	GTG	AAC	AGC	AGT	GAG	AGT	CTC	CCT	CCA	TCC	1357	
Ser	Pro	Ser	Gly	Ala	Ala	Val	Asn	Ser	Ser	Glu	Ser	Leu	Pro	Pro	Ser		
415					420					425					430		
TCG	TCT	GTC	AAT	GAC	ATC	TCC	TCC	ATG	TCC	ACC	GAC	CAG	ACC	CTG	GCA	1405	
Ser	Ser	Val	Asn	Asp	Ile	Ser	Ser	Met	Ser	Thr	Asp	Gln	Thr	Leu	Ala		
435					440					445							
TCT	GAC	ACT	GAC	AGC	AGC	CTG	GAA	GCC	TCG	GCA	GGA	CCC	CTG	GGT	TGT	1453	
Ser	Asp	Thr	Asp	Ser	Ser	Leu	Glu	Ala	Ser	Ala	Gly	Pro	Leu	Gly	Cys		
450					455					460							
TGC	AGG	TGACTAGCCG	CCTGCCTGCG			AAACCCAGCG			TTCTTCAGGA			GATGAT			1505		
Cys	Arg																

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 464 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

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

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

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

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2367 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:					
GAGAAATGGC	GTGGCAGGGG	ACCCAGCGAG	CCCAGAGGGA	TTTTGCCGCT	GCTTCCTCTA 60
CCCCTGTATT	TCACGCAGCT	CTCTAAATTG	ACTCAGCTCC	AGGCTAGTGT	GAGAAACACC 120
AACAGCAGGC	CCATCTCAGA	TCTTCACTAT	GGCAACTTAT	GCAAGAAACT	GTTGAATTAG 180
ACCCGTTTCC	TATAGATGAG	AAACCATACA	AGCTGTGGTA	TTTATGAGCC	TCCATTTCTT 240
ATACTACTGC	AGTGAACCAA	CATTGGATGT	GAAAATTGCC	TTTTGTCAGG	GATTTCGATAA 300
ACAAGTGGAT	GTGTCATATA	TTGCCAAACA	TTACAACATG	AGCAAAAGCA	AAGTTGACAA 360
CCAGTTCTAC	AGTGTGGAAG	TGGGAGACTC	AACCTTCACA	GTTCTCAAGC	GCTACCAGAA 420
TCTAAAGCCT	ATTGGCTCTG	GGGCTCAGGG	CATAGTTTGT	GCCGCGTATG	ATGCTGTCCT 480
TGACAGAAAT	GTGGCCATTA	AGAAGCTCAG	CAGACCCTTT	CAGAACCAAA	CACATGCCAA 540
GAGAGCGTAC	CGGGAGCTGG	TCCTCATGAA	GTGTGTGAAC	CATAAAAACA	TTATTAGTTT 600
ATTAAATGTC	TTCACACCCC	AGAAAACGCT	GGAGGAGTTC	CAAGATGTTT	ACTTAGTAAT 660
GGAAGTGATG	GATGCCAACT	TATGTCAAGT	GATTCAGATG	GAATTAGACC	ATGAGCGAAT 720
GTCTTACCTG	CTGTACCAA	TGTTGTGTGG	CATTAAGCAC	CTCCATTCTG	CTGGAATTAT 780
TCACAGGGAT	TTAAAACCAA	GTAACATTGT	AGTCAAGTCT	GATTGCACAT	TGAAAATCCT 840
GGACTTTGGA	CTGGCCAGGA	CAGCAGGCAC	AAGCTTCATG	ATGACTCCAT	ATGTGGTGAC 900
ACGTTATTAC	AGAGCCCCTG	AGGTCATCCT	GGGGATGGGC	TACAAGGAGA	ACGTGGATAT 960
ATGGTCTGTG	GGATGCATTA	TGGGAGAAAT	GGTTCGCCAC	AAAATCCTCT	TTCCAGGAAG 1020
GGACTATATT	GACCAGTGGA	ATAAGGTAAT	TGAACAAC TA	GGAACACCAT	GTCCAGAATT 1080
CATGAAGAAA	TTGCAACCCA	CAGTAAGAAA	CTATGTGGAG	AATCGGCCCA	AGTATGCGGG 1140
ACTCACCTTC	CCCAAAC TCT	TCCCAGATTC	CCTCTTCCCA	GCGGACTCCG	AGCACATAAA 1200
ACTCAAAGCC	AGCCAAGCCA	GGGACTTGTT	GTCAAAGATG	CTAGTGATTG	ACCCAGCAAA 1260
AAGAATATCA	GTGGACGACG	CCTTACAGCA	TCCCTACATC	AACGTCTGGT	ATGACCCAGC 1320
CGAAGTGGAG	GCGCCTCCAC	CTCAGATATA	TGACAAGCAG	TTGGATGAAA	GAGAACACAC 1380
AATTGAAGAA	TGAAAGAAC	TTATCTACAA	GGAAGTAATG	AATTCAGAAG	AAAAGACTAA 1440
AAATGGTGTA	GTAAAAGGAC	AGCCTTCTCC	TTCAGGTGCA	GCAGTGAACA	GCAGTGAGAG 1500
TCTCCCTCCA	TCCTCGTCTG	TCAATGACAT	CTCCTCCATG	TCCACCGACC	AGACCCTGGC 1560
ATCTGACACT	GACAGCAGCC	TGGAAGCCTC	GGCAGGACCC	CTGGGTTGTT	GCAGGTGACT 1620
AGCCGCTG C	CTGCGAAACC	CAGCGTTCTT	CAGGAGATGA	TGTGATGGAA	CACACACACA 1680
CGCAGACACA	CACACACACA	CAAATGCAGA	CACACAACAT	CAAGAAAACA	GCAAGGGAGA 1740
GAATCCAAGC	CTAAATTTAA	ATAAATCTTT	CAGCCTGCTT	CTTCCCCAGG	GTTCTGTATT 1800
GCAGCTAAGC	TCAAATGTAT	ATTTAACTTC	TAGTTGCTCT	TGCTTTGGTC	TTCTTCCAAT 1860
GATGCTTACT	ACAGAAAGCA	AATCAGACAC	AATTAGAGAA	GCCTTTTCCA	TAAAGTGTA A 1920
TTTTAATGGC	TGCAAAACCG	GCAACCTGTA	ACTGCCCTTT	TAAATGGCAT	GACAAGGTGT 1980
GCAGTGGCCC	CATCCAGCAT	GTGTGTGTCT	CTATCTTGCA	TCTACCTGCT	CCTTGGCCTA 2040
GTCAGATGGA	TGTAGATACA	GATCCGCATG	TGTCTGTATT	CATACAGCAC	TACTTACTTA 2100
GAGATGCTAC	TCTCAGTGTC	CTCAGGGCTC	TACCAAGACA	TAATGCACTG	GGGTACCACA 2160
TGGTCCATTT	CATGTGATCT	ATTACTCTGA	CATAAACCCA	TCTGTAATAT	ATTGCCAGTA 2220

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TATAAGCTGT TTAGTTTGTT AATTGATTAA ACTGTATGTC TTATAAGAAA ACATGTAAAG 2280
 GGGGAATATA TTGGGGGAGT GAGCTCTCTC AGACCCTTGA AGATGTAGCT TCCAAATTTG 2340
 AATGGATTAA ATGGCACCTG TATACCA 2367

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1773 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: Coding Sequence
 (B) LOCATION: 92...1357

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

ATCTCAGATC TTCACTATGG CAACTTATGC AAGAACTGT TGAATTAGAC CCGTTTCCTA 60
 TAGATGAGAA ACCATACAAG CTGTGGTATT T ATG AGC CTC CAT TTC TTA TAC 112
 Met Ser Leu His Phe Leu Tyr
 1 5
 TAC TGC AGT GAA CCA ACA TTG GAT GTG AAA ATT GCC TTT TGT CAG GGA 160
 Tyr Cys Ser Glu Pro Thr Leu Asp Val Lys Ile Ala Phe Cys Gln Gly
 10 15 20
 TTC GAT AAA CAA GTG GAT GTG TCA TAT ATT GCC AAA CAT TAC AAC ATG 208
 Phe Asp Lys Gln Val Asp Val Ser Tyr Ile Ala Lys His Tyr Asn Met
 25 30 35
 AGC AAA AGC AAA GTT GAC AAC CAG TTC TAC AGT GTG GAA GTG GGA GAC 256
 Ser Lys Ser Lys Val Asp Asn Gln Phe Tyr Ser Val Glu Val Gly Asp
 40 45 50 55
 TCA ACC TTC ACA GTT CTC AAG CGC TAC CAG AAT CTA AAG CCT ATT GGC 304
 Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Asn Leu Lys Pro Ile Gly
 60 65 70
 TCT GGG GCT CAG GGC ATA GTT TGT GCC GCG TAT GAT GCT GTC CTT GAC 352
 Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Tyr Asp Ala Val Leu Asp
 75 80 85
 AGA AAT GTG GCC ATT AAG AAG CTC AGC AGA CCC TTT CAG AAC CAA ACA 400
 Arg Asn Val Ala Ile Lys Lys Leu Ser Arg Pro Phe Gln Asn Gln Thr
 90 95 100
 CAT GCC AAG AGA GCG TAC CGG GAG CTG GTC CTC ATG AAG TGT GTG AAC 448
 His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Met Lys Cys Val Asn
 105 110 115
 CAT AAA AAC ATT ATT AGT TTA TTA AAT GTC TTC ACA CCC CAG AAA ACG 496
 His Lys Asn Ile Ile Ser Leu Leu Asn Val Phe Thr Pro Gln Lys Thr
 120 125 130 135
 CTG GAG GAG TTC CAA GAT GTT TAC TTA GTA ATG GAA CTG ATG GAT GCC 544
 Leu Glu Glu Phe Gln Asp Val Tyr Leu Val Met Glu Leu Met Asp Ala
 140 145 150
 AAC TTA TGT CAA GTG ATT CAG ATG GAA TTA GAC CAT GAG CGA ATG TCT 592
 Asn Leu Cys Gln Val Ile Gln Met Glu Leu Asp His Glu Arg Met Ser
 155 160 165
 TAC CTG CTG TAC CAA ATG TTG TGT GGC ATT AAG CAC CTC CAT TCT GCT 640
 Tyr Leu Leu Tyr Gln Met Leu Cys Gly Ile Lys His Leu His Ser Ala
 170 175 180
 GGA ATT ATT CAC AGG GAT TTA AAA CCA AGT AAC ATT GTA GTC AAG TCT 688
 Gly Ile Ile His Arg Asp Leu Lys Pro Ser Asn Ile Val Val Lys Ser

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185						190						195					
GAT	TGC	ACA	TTG	AAA	ATC	CTG	GAC	TTT	GGA	CTG	GCC	AGG	ACA	GCA	GGC		736
Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala	Gly		
200					205				210						215		
ACA	AGC	TTC	ATG	ATG	ACT	CCA	TAT	GTG	GTG	ACA	CGT	TAT	TAC	AGA	GCC		784
Thr	Ser	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg	Ala		
			220					225						230			
CCT	GAG	GTC	ATC	CTG	GGG	ATG	GGC	TAC	AAG	GAG	AAC	GTG	GAT	ATA	TGG		832
Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile	Trp		
			235					240						245			
TCT	GTG	GGA	TGC	ATT	ATG	GGA	GAA	ATG	GTT	CGC	CAC	AAA	ATC	CTC	TTT		880
Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Met	Val	Arg	His	Lys	Ile	Leu	Phe		
		250					255					260					
CCA	GGA	AGG	GAC	TAT	ATT	GAC	CAG	TGG	AAT	AAG	GTA	ATT	GAA	CAA	CTA		928
Pro	Gly	Arg	Asp	Tyr	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln	Leu		
	265					270					275						
GGA	ACA	CCA	TGT	CCA	GAA	TTC	ATG	AAG	AAA	TTG	CAA	CCC	ACA	GTA	AGA		976
Gly	Thr	Pro	Cys	Pro	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	Thr	Val	Arg		
280					285					290					295		
AAC	TAT	GTG	GAG	AAT	CGG	CCC	AAG	TAT	GCG	GGA	CTC	ACC	TTC	CCC	AAA		1024
Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Ala	Gly	Leu	Thr	Phe	Pro	Lys		
				300				305						310			
CTC	TTC	CCA	GAT	TCC	CTC	TTC	CCA	GCG	GAC	TCC	GAG	CAC	AAT	AAA	CTC		1072
Leu	Phe	Pro	Asp	Ser	Leu	Phe	Pro	Ala	Asp	Ser	Glu	His	Asn	Lys	Leu		
			315					320					325				
AAA	GCC	AGC	CAA	GCC	AGG	GAC	TTG	TTG	TCA	AAG	ATG	CTA	GTG	ATT	GAC		1120
Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile	Asp		
		330					335					340					
CCA	GCA	AAA	AGA	ATA	TCA	GTG	GAC	GAC	GCC	TTA	CAG	CAT	CCC	TAC	ATC		1168
Pro	Ala	Lys	Arg	Ile	Ser	Val	Asp	Asp	Ala	Leu	Gln	His	Pro	Tyr	Ile		
	345					350					355						
AAC	GTC	TGG	TAT	GAC	CCA	GCC	GAA	GTG	GAG	GCG	CCT	CCA	CCT	CAG	ATA		1216
Asn	Val	Trp	Tyr	Asp	Pro	Ala	Glu	Val	Glu	Ala	Pro	Pro	Pro	Gln	Ile		
360					365					370					375		
TAT	GAC	AAG	CAG	TTG	GAT	GAA	AGA	GAA	CAC	ACA	ATT	GAA	GAA	TGG	AAA		1264
Tyr	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu	His	Thr	Ile	Glu	Glu	Trp	Lys		
				380				385						390			
GAA	CTT	ATC	TAC	AAG	GAA	GTA	ATG	AAT	TCA	GAA	GAA	AAG	ACT	AAA	AAT		1312
Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asn	Ser	Glu	Glu	Lys	Thr	Lys	Asn		
			395					400					405				
GGT	GTA	GTA	AAA	GGA	CAG	CCT	TCT	CCT	TCA	GCA	CAG	GTG	CAG	CAG			1357
Gly	Val	Val	Lys	Gly	Gln	Pro	Ser	Pro	Ser	Ala	Gln	Val	Gln	Gln			
	410					415						420					
TGAACAGCAG	TGAGAGTCTC	CCTCCATCCT	CGTCTGTCAA	TGACATCTCC	TCCATGTCCA												1417
CCGACCAGAC	CCTGGCATCT	GACACTGACA	GCAGCCTGGA	AGCCTCGGCA	GGACCCCTGG												1477
GTTGTTGCAG	GTGACTAGCC	GCCTGCCTGC	GAAACCCAGC	GTTCTTCAGG	AGATGATGTG												1537
ATGGAACACA	CACACACGCA	GACACACACA	CACACACAAA	TGCAGACACA	CAACATCAAG												1597
AAAACAGCAA	GGGAGAGAAT	CCAAGCCTAA	AATTAAATAA	ATCTTTCAGC	CTGCTTCTTC												1657
CCCAGGGTTC	TGTATTGCAG	CTAAGCTCAA	ATGTATATTT	AACTTCTAGT	TGCTCTTGCT												1717
TTGGTCTTCT	TCCAATGATG	CTTACTACAG	AAAGCAAATC	AGACACAATT	AGAGAA												1773

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 422 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

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

Met	Ser	Leu	His	Phe	Leu	Tyr	Tyr	Cys	Ser	Glu	Pro	Thr	Leu	Asp	Val
1				5					10					15	
Lys	Ile	Ala	Phe	Cys	Gln	Gly	Phe	Asp	Lys	Gln	Val	Asp	Val	Ser	Tyr
			20					25					30		
Ile	Ala	Lys	His	Tyr	Asn	Met	Ser	Lys	Ser	Lys	Val	Asp	Asn	Gln	Phe
		35					40					45			
Tyr	Ser	Val	Glu	Val	Gly	Asp	Ser	Thr	Phe	Thr	Val	Leu	Lys	Arg	Tyr
	50					55					60				
Gln	Asn	Leu	Lys	Pro	Ile	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala
65					70					75					80
Ala	Tyr	Asp	Ala	Val	Leu	Asp	Arg	Asn	Val	Ala	Ile	Lys	Lys	Leu	Ser
				85					90					95	
Arg	Pro	Phe	Gln	Asn	Gln	Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu
		100						105					110		
Val	Leu	Met	Lys	Cys	Val	Asn	His	Lys	Asn	Ile	Ile	Ser	Leu	Leu	Asn
	115					120						125			
Val	Phe	Thr	Pro	Gln	Lys	Thr	Leu	Glu	Glu	Phe	Gln	Asp	Val	Tyr	Leu
	130					135					140				
Val	Met	Glu	Leu	Met	Asp	Ala	Asn	Leu	Cys	Gln	Val	Ile	Gln	Met	Glu
145					150					155				160	
Leu	Asp	His	Glu	Arg	Met	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Cys	Gly
			165						170					175	
Ile	Lys	His	Leu	His	Ser	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro
		180						185					190		
Ser	Asn	Ile	Val	Val	Lys	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe
	195						200					205			
Gly	Leu	Ala	Arg	Thr	Ala	Gly	Thr	Ser	Phe	Met	Met	Thr	Pro	Tyr	Val
	210					215					220				
Val	Thr	Arg	Tyr	Tyr	Arg	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr
225					230					235				240	
Lys	Glu	Asn	Val	Asp	Ile	Trp	Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Met
			245						250					255	
Val	Arg	His	Lys	Ile	Leu	Phe	Pro	Gly	Arg	Asp	Tyr	Ile	Asp	Gln	Trp
		260						265					270		
Asn	Lys	Val	Ile	Glu	Gln	Leu	Gly	Thr	Pro	Cys	Pro	Glu	Phe	Met	Lys
		275					280					285			
Lys	Leu	Gln	Pro	Thr	Val	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr
	290					295					300				
Ala	Gly	Leu	Thr	Phe	Pro	Lys	Leu	Phe	Pro	Asp	Ser	Leu	Phe	Pro	Ala
305					310					315				320	
Asp	Ser	Glu	His	Asn	Lys	Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu
			325						330					335	
Ser	Lys	Met	Leu	Val	Ile	Asp	Pro	Ala	Lys	Arg	Ile	Ser	Val	Asp	Asp
		340						345					350		

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Ala	Leu	Gln	His	Pro	Tyr	Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ala	Glu	Val
	355						360					365			
Glu	Ala	Pro	Pro	Pro	Gln	Ile	Tyr	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu
	370					375					380				
His	Thr	Ile	Glu	Glu	Trp	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asn
385					390					395					400
Ser	Glu	Glu	Lys	Thr	Lys	Asn	Gly	Val	Val	Lys	Gly	Gln	Pro	Ser	Pro
				405					410					415	
Ser	Ala	Gln	Val	Gln	Gln										
				420											

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2372 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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

GAGAAATGGC	GTGGCAGGGG	ACCCAGCGAG	CCCAGAGGGA	TTTGTCCGCT	GCTTCCTCTA	60
CCCCTGTATT	TCACGCAGCT	CTCTAAATTG	ACTCAGCTCC	AGGCTAGTGT	GAGAAACACC	120
AACAGCAGGC	CCATCTCAGA	TCTTCACTAT	GGCAACTTAT	GCAAGAAACT	GTTGAATTAG	180
ACCCGTTTCC	TATAGATGAG	AAACCATACA	AGCTGTGGTA	TTTATGAGCC	TCCATTTCTT	240
ATACTACTGC	AGTGAACCAA	CATTGGATGT	GAAAATTGCC	TTTTGTCAGG	GATTCGATAA	300
ACAAGTGGAT	GTGTCATATA	TTGCCAAACA	TTACAACATG	AGCAAAAGCA	AAGTTGACAA	360
CCAGTTCTAC	AGTGTGGAAG	TGGGAGACTC	AACCTTCACA	GTTCTCAAGC	GCTACCAGAA	420
TCTAAAGCCT	ATTGGCTCTG	GGGCTCAGGG	CATAGTTTGT	GCCGCGTATG	ATGCTGTCCT	480
TGACAGAAAT	GTGGCCATTA	AGAAGCTCAG	CAGACCCTTT	CAGAACCAAA	CACATGCCAA	540
GAGAGCGTAC	CGGGAGCTGG	TCCTCATGAA	GTGTGTGAAC	CATAAAAACA	TTATTAGTTT	600
ATTAAATGTC	TTACACCCCC	AGAAAACGCT	GGAGGAGTTC	CAAGATGTTT	ACTTAGTAAT	660
GGAAGTGATG	GATGCCAACT	TATGTCAAGT	GATTCAGATG	GAATTAGACC	ATGAGCGAAT	720
GTCTTACCTG	CTGTACCAAA	TGTTGTGTGG	CATTAAGCAC	CTCCATTCTG	CTGGAATTAT	780
TCACAGGGAT	TTAAAACCAA	GTAACATTGT	AGTCAAGTCT	GATTGCACAT	TGAAAATCCT	840
GGACTTTGGA	CTGGCCAGGA	CAGCAGGCAC	AAGCTTCATG	ATGACTCCAT	ATGTGGTGAC	900
ACGTTATTAC	AGAGCCCCTG	AGGTCATCCT	GGGGATGGGC	TACAAGGAGA	ACGTGGATAT	960
ATGGTCTGTG	GGATGCATTA	TGGGAGAAAT	GGTTCGCCAC	AAAATCCTCT	TTCCAGGAAG	1020
GGACTATATT	GACCAGTGGA	ATAAGGTAAT	TGAACAAC TA	GGAACACCAT	GTCCAGAATT	1080
CATGAAGAAA	TTGCAACCCA	CAGTAAGAAA	CTATGTGGAG	AATCGGCCCA	AGTATGCGGG	1140
ACTCACCTTC	CCCAAAC TCT	TCCCAGATT C	CCTCTTCCCA	GCGGACTCCG	AGCACAATAA	1200
ACTCAAAGCC	AGCCAAGCCA	GGGACTTGTT	GTCAAAGATG	CTAGTGATTG	ACCCAGCAAA	1260
AAGAATATCA	GTGGACGACG	CCTTACAGCA	TCCCTACATC	AACGTCTGGT	ATGACCCAGC	1320
CGAAGTGGAG	GCGCCTCCAC	CTCAGATATA	TGACAAGCAG	TTGGATGAAA	GAGAACACAC	1380
AATTGAAGAA	TGGAAGAAC	TTATCTACAA	GGAAGTAATG	AATTCAGAAG	AAAAGACTAA	1440

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AAATGGTGTA	GTAAAAGGAC	AGCCTTCTCC	TTCAGCACAG	GTGCAGCAGT	GAACAGCAGT	1500
GAGAGTCTCC	CTCCATCCTC	GTCTGTCAAT	GACATCTCCT	CCATGTCCAC	CGACCAGACC	1560
CTGGCATCTG	ACACTGACAG	CAGCCTGGAA	GCCTCGGCAG	GACCCCTGGG	TTGTTGCAGG	1620
TGACTAGCCG	CCTGCCTGCG	AAACCCAGCG	TTCTTCAGGA	GATGATGTGA	TGGAACACAC	1680
ACACACGCAG	ACACACACAC	ACACACAAAT	GCAGACACAC	AACATCAAGA	AAACAGCAAG	1740
GGAGAGAATC	CAAGCCTAAA	ATTAAATAAA	TCTTTCAGCC	TGCTTCTTCC	CCAGGGTTCT	1800
GTATTGCAGC	TAAGCTCAAA	TGTATATTTA	ACTTCTAGTT	GCTCTTGCTT	TGGTCTTCTT	1860
CCAATGATGC	TTACTACAGA	AAGCAAATCA	GACACAATTA	GAGAAGCCTT	TTCCATAAAG	1920
TGTAATTTTA	ATGGCTGCAA	AACCGGCAAC	CTGTAAGTGC	CCTTTTAAAT	GGCATGACAA	1980
GGTGTGCAGT	GGCCCCATCC	AGCATGTGTG	TGTCTCTATC	TTGCATCTAC	CTGCTCCTTG	2040
GCCTAGTCAG	ATGGATGTAG	ATACAGATCC	GCATGTGTCT	GTATTCATAC	AGCACTACTT	2100
ACTTAGAGAT	GCTACTCTCA	GTGTCCTCAG	GGCTCTACCA	AGACATAATG	CACTGGGGTA	2160
CCACATGGTC	CATTTCATGT	GATCTATTAC	TCTGACATAA	ACCCATCTGT	AATATATTGC	2220
CAGTATATAA	GCTGTTTAGT	TTGTTAATTG	ATTAAACTGT	ATGTCTTATA	AGAAAACATG	2280
TAAAGGGGGA	ATATATTGGG	GGAGTGAGCT	CTCTCAGACC	CTTGAAGATG	TAGCTTCCAA	2340
ATTTGAATGG	ATTAAATGGC	ACCTGTATAC	CA			2372

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2372 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 - (A) NAME/KEY: Coding Sequence
 - (B) LOCATION: 224...1489

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

GAGAAATGGC	GTGGCAGGGG	ACCCAGCGAG	CCCAGAGGGA	TTTTGCCGCT	GCTTCCTCTA	60
CCCCTGTATT	TCACGCAGCT	CTCTAAATTG	ACTCAGCTCC	AGGCTAGTGT	GAGAAACACC	120
AACAGCAGGC	CCATCTCAGA	TCTTCACTAT	GGCAACTTAT	GCAAGAAACT	GTTGAATTAG	180
ACCCGTTTCC	TATAGATGAG	AAACCATACA	AGCTGTGGTA	TTT ATG AGC CTC CAT		235
				Met Ser Leu His		
				1		
TTC TTA TAC TAC TGC AGT GAA CCA ACA TTG GAT GTG AAA ATT GCC TTT						283
Phe Leu Tyr Tyr Cys Ser Glu Pro Thr Leu Asp Val Lys Ile Ala Phe						
5 10 15 20						
TGT CAG GGA TTC GAT AAA CAA GTG GAT GTG TCA TAT ATT GCC AAA CAT						331
Cys Gln Gly Phe Asp Lys Gln Val Asp Val Ser Tyr Ile Ala Lys His						
25 30 35						
TAC AAC ATG AGC AAA AGC AAA GTT GAC AAC CAG TTC TAC AGT GTG GAA						379
Tyr Asn Met Ser Lys Ser Lys Val Asp Asn Gln Phe Tyr Ser Val Glu						
40 45 50						
GTG GGA GAC TCA ACC TTC ACA GTT CTC AAG CGC TAC CAG AAT CTA AAG						427
Val Gly Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Asn Leu Lys						
55 60 65						
CCT ATT GGC TCT GGG GCT CAG GGC ATA GTT TGT GCC GCG TAT GAT GCT						475

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Pro	Ile	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala	Ala	Tyr	Asp	Ala	
	70					75					80					
GTC	CTT	GAC	AGA	AAT	GTG	GCC	ATT	AAG	AAG	CTC	AGC	AGA	CCC	TTT	CAG	523
Val	Leu	Asp	Arg	Asn	Val	Ala	Ile	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	
85					90					95					100	
AAC	CAA	ACA	CAT	GCC	AAG	AGA	GCG	TAC	CGG	GAG	CTG	GTC	CTC	ATG	AAG	571
Asn	Gln	Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu	Val	Leu	Met	Lys	
				105					110					115		
TGT	GTG	AAC	CAT	AAA	AAC	ATT	ATT	AGT	TTA	TTA	AAT	GTC	TTC	ACA	CCC	619
Cys	Val	Asn	His	Lys	Asn	Ile	Ile	Ser	Leu	Leu	Asn	Val	Phe	Thr	Pro	
			120					125					130			
CAG	AAA	ACG	CTG	GAG	GAG	TTC	CAA	GAT	GTT	TAC	TTA	GTA	ATG	GAA	CTG	667
Gln	Lys	Thr	Leu	Glu	Glu	Phe	Gln	Asp	Val	Tyr	Leu	Val	Met	Glu	Leu	
		135					140					145				
ATG	GAT	GCC	AAC	TTA	TGT	CAA	GTG	ATT	CAG	ATG	GAA	TTA	GAC	CAT	GAG	715
Met	Asp	Ala	Asn	Leu	Cys	Gln	Val	Ile	Gln	Met	Glu	Leu	Asp	His	Glu	
	150					155					160					
CGA	ATG	TCT	TAC	CTG	CTG	TAC	CAA	ATG	TTG	TGT	GGC	ATT	AAG	CAC	CTC	763
Arg	Met	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Cys	Gly	Ile	Lys	His	Leu	
165					170					175					180	
CAT	TCT	GCT	GGA	ATT	ATT	CAC	AGG	GAT	TTA	AAA	CCA	AGT	AAC	ATT	GTA	811
His	Ser	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Ile	Val	
				185					190					195		
GTC	AAG	TCT	GAT	TGC	ACA	TTG	AAA	ATC	CTG	GAC	TTT	GGA	CTG	GCC	AGG	859
Val	Lys	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	
			200					205					210			
ACA	GCA	GGC	ACA	AGC	TTC	ATG	ATG	ACT	CCA	TAT	GTG	GTG	ACA	CGT	TAT	907
Thr	Ala	Gly	Thr	Ser	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	
		215					220					225				
TAC	AGA	GCC	CCT	GAG	GTC	ATC	CTG	GGG	ATG	GGC	TAC	AAG	GAG	AAC	GTG	955
Tyr	Arg	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	
	230					235					240					
GAT	ATA	TGG	TCT	GTG	GGA	TGC	ATT	ATG	GGA	GAA	ATG	GTT	CGC	CAC	AAA	1003
Asp	Ile	Trp	Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Met	Val	Arg	His	Lys	
245				250						255					260	
ATC	CTC	TTT	CCA	GGA	AGG	GAC	TAT	ATT	GAC	CAG	TGG	AAT	AAG	GTA	ATT	1051
Ile	Leu	Phe	Pro	Gly	Arg	Asp	Tyr	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	
				265					270					275		
GAA	CAA	CTA	GGA	ACA	CCA	TGT	CCA	GAA	TTC	ATG	AAG	AAA	TTG	CAA	CCC	1099
Glu	Gln	Leu	Gly	Thr	Pro	Cys	Pro	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	
			280					285					290			
ACA	GTA	AGA	AAC	TAT	GTG	GAG	AAT	CGG	CCC	AAG	TAT	GCG	GGA	CTC	ACC	1147
Thr	Val	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Ala	Gly	Leu	Thr	
			295				300					305				
TTC	CCC	AAA	CTC	TTC	CCA	GAT	TCC	CTC	TTC	CCA	GCG	GAC	TCC	GAG	CAC	1195
Phe	Pro	Lys	Leu	Phe	Pro	Asp	Ser	Leu	Phe	Pro	Ala	Asp	Ser	Glu	His	
	310					315					320					
AAT	AAA	CTC	AAA	GCC	AGC	CAA	GCC	AGG	GAC	TTG	TTG	TCA	AAG	ATG	CTA	1243
Asn	Lys	Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	
325				330					335					340		
GTG	ATT	GAC	CCA	GCA	AAA	AGA	ATA	TCA	GTG	GAC	GAC	GCC	TTA	CAG	CAT	1291
Val	Ile	Asp	Pro	Ala	Lys	Arg	Ile	Ser	Val	Asp	Asp	Ala	Leu	Gln	His	
				345					350					355		
CCC	TAC	ATC	AAC	GTC	TGG	TAT	GAC	CCA	GCC	GAA	GTG	GAG	GCG	CCT	CCA	1339
Pro	Tyr	Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ala	Glu	Val	Glu	Ala	Pro	Pro	
			360				365						370			
CCT	CAG	ATA	TAT	GAC	AAG	CAG	TTG	GAT	GAA	AGA	GAA	CAC	ACA	ATT	GAA	1387

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Pro	Gln	Ile	Tyr	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu	His	Thr	Ile	Glu		
	375						380					385					
GAA	TGG	AAA	GAA	CTT	ATC	TAC	AAG	GAA	GTA	ATG	AAT	TCA	GAA	GAA	AAG	1435	
Glu	Trp	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asn	Ser	Glu	Glu	Lys		
	390						395				400						
ACT	AAA	AAT	GGT	GTA	GTA	AAA	GGA	CAG	CCT	TCT	CCT	TCA	GCA	CAG	GTG	1483	
Thr	Lys	Asn	Gly	Val	Val	Lys	Gly	Gln	Pro	Ser	Pro	Ser	Ala	Gln	Val		
405					410				415					420			
CAG	CAG	TGAACAGCAG	TGAGAGTCTC	CCTCCATCCT	CGTCTGTCAA	TGACATCTCC										1539	
Gln	Gln																
TCCATGTCCA	CCGACCAGAC	CCTGGCATCT	GACACTGACA	GCAGCCTGGA	AGCCTCGGCA											1599	
GGACCCCTGG	GTGTGTCAG	GTGACTAGCC	GCCTGCCTGC	GAAACCCAGC	GTTCTTCAGG											1659	
AGATGATGTG	ATGGAACACA	CACACACGCA	GACACACACA	CACACACAAA	TGCAGACACA											1719	
CAACATCAAG	AAAACAGCAA	GGGAGAGAAT	CCAAGCCTAA	AATTAAATAA	ATCTTTCAGC											1779	
CTGCTTCTTC	CCCAGGGTTC	TGTATTGCAG	CTAAGCTCAA	ATGTATATTT	AACTTCTAGT											1839	
TGCTCTTGCT	TTGGTCTTCT	TCCAATGATG	CTTACTACAG	AAAGCAAATC	AGACACAATT											1899	
AGAGAAGCCT	TTTCCATAAA	GTGTAATTTT	AATGGCTGCA	AAACCGGCAA	CCTGTAACTG											1959	
CCCTTTTAAA	TGGCATGACA	AGGTGTGCAG	TGGCCCCATC	CAGCATGTGT	GTGTCTCTAT											2019	
CTTGCACTTA	CCTGCTCCTT	GGCCTAGTCA	GATGGATGTA	GATACAGATC	CGCATGTGTC											2079	
TGTATTCATA	CAGCACTACT	TACTTAGAGA	TGCTACTCTC	AGTGTCTTCA	GGGCTCTACC											2139	
AAGACATAAT	GCACTGGGGT	ACCACATGGT	CCATTTTCATG	TGATCTATTA	CTCTGACATA											2199	
AACCCATCTG	TAATATATTG	CCAGTATATA	AGCTGTTTAG	TTTGTTAATT	GATTAAACTG											2259	
TATGTCTTAT	AAGAAAACAT	GTAAAGGGGG	AATATATTGG	GGGAGTGAGC	TCTCTCAGAC											2319	
CCTTGAAGAT	GTAGCTTCCA	AATTTGAATG	GATTAAATGG	CACCTGTATA	CCA											2372	

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 422 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

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

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

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

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1975 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (ix) FEATURE:
 - (A) NAME/KEY: Coding Sequence
 - (B) LOCATION: 364...1641

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

CCCTCCTTAT TCCGGTTTGG AATGTGGCTA ATGAAAGCCC AGTAGGAGGA TTTCTGGGGC	60
AAACAGGTGG ACCAGGATCC TGGTTCTCAG GCACGGAATG GCTATTGTGA GAGCGCCACC	120
AGCAGGACCA TCGCAGATCT TGGTTATGGC TGCTCACGCA AGAGGCTGTT GATGTAGACC	180
CCCTTTCCCG TAGATGAGAA ATCACACGAG CAGTGGTATT TATGAGCCTC CATTTCTTAT	240
ACTACTGCAG TGAACCAACC TTGGATGTGA AAATTGCCTT TTGTCAGGTG TGTGTTCTT	300
ACAGGTAAAA CAAAGGGATT CGACAAACAC GTGGATGTGT CTTCTGTTGT CAAACATTAC	360
AAC ATG AGC AAA AGC AAG GTA GAT AAC CAG TTC TAC AGT GTG GAA GTG Met Ser Lys Ser Lys Val Asp Asn Gln Phe Tyr Ser Val Glu Val 1 5 10 15	408
GGA GAC TCA ACC TTC ACA GTT CTA AAG CGC TAC CAG AAC CTG AAG CCG Gly Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Asn Leu Lys Pro 20 25 30	456
ATC GGC TCT GGG GCT CAG GGA ATA GTT TGT GCT GCG TAT GAC GCT GTC Ile Gly Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Tyr Asp Ala Val 35 40 45	504
CTC GAC AGA AAT GTG GCC ATT AAG AAG CTC AGC AGA CCC TTC CAG AAC Leu Asp Arg Asn Val Ala Ile Lys Lys Leu Ser Arg Pro Phe Gln Asn 50 55 60	552
CAA ACT CAT GCC AAG AGG GCT TAC CGG GAG CTG GTC CTC ATG AAG TGT Gln Thr His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Met Lys Cys 65 70 75	600
GTG AAC CAT AAA AAC ATT ATT AGC TTA TTA AAT GTC TTT ACA CCC CAG Val Asn His Lys Asn Ile Ile Ser Leu Leu Asn Val Phe Thr Pro Gln 80 85 90 95	648
AAA ACA CTG GAG GAG TTC CAA GAT GTT TAC TTA GTG ATG GAA CTG ATG Lys Thr Leu Glu Phe Gln Asp Val Tyr Leu Val Met Glu Leu Met 100 105 110	696
GAC GCC AAC TTG TGT CAG GTG ATT CAG ATG GAG CTG GAC CAC GAG CGG Asp Ala Asn Leu Cys Gln Val Ile Gln Met Glu Leu Asp His Glu Arg 115 120 125	744
ATG TCG TAC TTG CTG TAC CAG ATG CTG TCG GCG ATC AAA CAC CTC CAC Met Ser Tyr Leu Leu Tyr Gln Met Leu Ser Ala Ile Lys His Leu His 130 135 140	792
TCC GCT GGG ATC ATC CAC AGG GAC TTA AAA CCC AGT AAC ATC GTA GTC Ser Ala Gly Ile Ile His Arg Asp Leu Lys Pro Ser Asn Ile Val Val 145 150 155	840
AAG TCT GAT TGC ACA CTG AAA ATC CTG GAC TTT GGA CTG GCC AGG ACA Lys Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg Thr 160 165 170 175	888
GCG GGC ACA AGC TTC ATG ATG ACT CCG TAT GTG GTG ACG AGA TAT TAC Ala Gly Thr Ser Phe Met Met Thr Pro Tyr Val Val Thr Arg Tyr Tyr 180 185 190	936
AGA GCC CCC GAG GTC ATC CTG GGC ATG GGC TAC AAG GAG AAC GTG GAC Arg Ala Pro Glu Val Ile Leu Gly Met Gly Tyr Lys Glu Asn Val Asp 195 200 205	984
ATA TGG TCT GTG GGC TGC ATC ATG GGA GAA ATG GTT CGT CAC AAA ATC Ile Trp Ser Val Gly Cys Ile Met Gly Glu Met Val Arg His Lys Ile 210 215 220	1032
CTC TTT CCC GGA AGG GAC TAT ATT GAC CAG TGG AAC AAA GTC ATA GAG Leu Phe Pro Gly Arg Asp Tyr Ile Asp Gln Trp Asn Lys Val Ile Glu 225 230 235	1080
CAG CTA GGA ACT CCG TGT CCA GAA TTC ATG AAG AAA TTG CAG CCC ACC Gln Leu Gly Thr Pro Cys Pro Glu Phe Met Lys Lys Leu Gln Pro Thr	1128

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240				245				250				255						
GTC	AGA	AAC	TAC	GTG	GAG	AAC	CGG	CCC	AAG	TAT	GCA	GGC	CTC	ACC	TTC	1176		
Val	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Ala	Gly	Leu	Thr	Phe			
260				265				270										
CCC	AAG	CTC	TTT	CCA	GAT	TCC	CTC	TTC	CCA	GCG	GAT	TCC	GAG	CAC	AAT	1224		
Pro	Lys	Leu	Phe	Pro	Asp	Ser	Leu	Phe	Pro	Ala	Asp	Ser	Glu	His	Asn			
275				280				285										
AAA	CTT	AAA	GCC	AGC	CAA	GCC	AGG	GAC	TTG	TTG	TCA	AAG	ATG	TTA	GTG	1272		
Lys	Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val			
290				295				300										
ATT	GAC	CCA	GCG	AAG	AGG	ATA	TCG	GTG	GAT	GAC	GCA	TTG	CAG	CAT	CCG	1320		
Ile	Asp	Pro	Ala	Lys	Arg	Ile	Ser	Val	Asp	Asp	Ala	Leu	Gln	His	Pro			
305				310				315										
TAC	ATC	AAC	GTT	TGG	TAC	GAC	CCT	GCT	GAA	GTG	GAG	GCG	CCT	CCG	CCT	1368		
Tyr	Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ala	Glu	Val	Glu	Ala	Pro	Pro	Pro			
320				325				330				335						
CAG	ATA	TAT	GAC	AAG	CAA	TTG	GAT	GAA	AGG	GAG	CAC	ACC	ATC	GAA	GAA	1416		
Gln	Ile	Tyr	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu	His	Thr	Ile	Glu	Glu			
340				345				350										
TGG	AAA	GAA	CTC	ATC	TAC	AAG	GAA	GTA	ATG	AAC	TCA	GAA	GAG	AAG	ACT	1464		
Trp	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asn	Ser	Glu	Glu	Lys	Thr			
355				360				365										
AAG	AAC	GGC	GTA	GTC	AAA	GGC	CAG	CCC	TCA	CCT	TCA	GGT	GCA	GCA	GTG	1512		
Lys	Asn	Gly	Val	Val	Lys	Gly	Gln	Pro	Ser	Pro	Ser	Gly	Ala	Ala	Val			
370				375				380										
AAC	AGC	AGT	GAG	AGT	CTC	CCT	CCA	TCC	TCA	TCT	GTC	AAC	GAC	ATC	TCC	1560		
Asn	Ser	Ser	Glu	Ser	Leu	Pro	Pro	Ser	Ser	Ser	Val	Asn	Asp	Ile	Ser			
385				390				395										
TCC	ATG	TCC	ACC	GAC	CAG	ACC	CTC	GCA	TCC	GAC	ACT	GAC	AGC	AGC	CTG	1608		
Ser	Met	Ser	Thr	Asp	Gln	Thr	Leu	Ala	Ser	Asp	Thr	Asp	Ser	Ser	Leu			
400				405				410				415						
GAA	GCC	TCG	GCG	GGA	CCG	CTG	GGT	TGT	TGC	AGG	TGACTAGCCG	CCTGCCTGCG				1661		
Glu	Ala	Ser	Ala	Gly	Pro	Leu	Gly	Cys	Cys	Arg								
420				425														
AAACCCAGCG TTCTTCAGGA GATGACGCCA TGATAGAACA CAGCGCACAT GCACACACAC																		1721
AGAGCTTGTA CACACACACA CACACACACA CACACGCACG CACGCACGCA CGCAAGCACG																		1781
CACGCACGCA CAAATGCACT CACGCAATGT CAAGAAAAAA AAAAGTAGCG AGAGAGAGCG																		1841
AGAGAGCCAA CGTAAACTA AGTTAAATCT TTCTGCGTGC TTCTCCAGAG TTCTGTATCG																		1901
CAGCTGAGCT GAAATGTATA CTTAACTTCT AGTCGCGCTC GCTCGACTTT GGTCTCCCTC																		1961
CGGCAGTGCT TACT																		1975

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 426 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

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

Met Ser Lys Ser Lys Val Asp Asn Gln Phe Tyr Ser Val Glu Val Gly
1 5 10 15

Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Asn Leu Lys Pro Ile

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20				25				30							
Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala	Ala	Tyr	Asp	Ala	Val	Leu
		35					40					45			
Asp	Arg	Asn	Val	Ala	Ile	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	Asn	Gln
	50					55					60				
Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu	Val	Leu	Met	Lys	Cys	Val
	65				70					75					80
Asn	His	Lys	Asn	Ile	Ile	Ser	Leu	Leu	Asn	Val	Phe	Thr	Pro	Gln	Lys
			85						90					95	
Thr	Leu	Glu	Glu	Phe	Gln	Asp	Val	Tyr	Leu	Val	Met	Glu	Leu	Met	Asp
			100					105					110		
Ala	Asn	Leu	Cys	Gln	Val	Ile	Gln	Met	Glu	Leu	Asp	His	Glu	Arg	Met
		115					120					125			
Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Ser	Ala	Ile	Lys	His	Leu	His	Ser
	130					135					140				
Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Ile	Val	Val	Lys
145					150					155					160
Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala
			165						170					175	
Gly	Thr	Ser	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg
		180						185					190		
Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile
		195					200					205			
Trp	Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Met	Val	Arg	His	Lys	Ile	Leu
	210					215					220				
Phe	Pro	Gly	Arg	Asp	Tyr	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln
225					230					235					240
Leu	Gly	Thr	Pro	Cys	Pro	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	Thr	Val
			245						250					255	
Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Ala	Gly	Leu	Thr	Phe	Pro
		260						265					270		
Lys	Leu	Phe	Pro	Asp	Ser	Leu	Phe	Pro	Ala	Asp	Ser	Glu	His	Asn	Lys
		275					280					285			
Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile
	290					295					300				
Asp	Pro	Ala	Lys	Arg	Ile	Ser	Val	Asp	Asp	Ala	Leu	Gln	His	Pro	Tyr
305					310					315					320
Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ala	Glu	Val	Glu	Ala	Pro	Pro	Pro	Gln
			325						330					335	
Ile	Tyr	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu	His	Thr	Ile	Glu	Glu	Trp
		340						345					350		
Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asn	Ser	Glu	Glu	Lys	Thr	Lys
		355					360					365			
Asn	Gly	Val	Val	Lys	Gly	Gln	Pro	Ser	Pro	Ser	Gly	Ala	Ala	Val	Asn
	370					375					380				
Ser	Ser	Glu	Ser	Leu	Pro	Pro	Ser	Ser	Ser	Val	Asn	Asp	Ile	Ser	Ser
385					390					395					400
Met	Ser	Thr	Asp	Gln	Thr	Leu	Ala	Ser	Asp	Thr	Asp	Ser	Ser	Leu	Glu
			405						410					415	
Ala	Ser	Ala	Gly	Pro	Leu	Gly	Cys	Cys	Arg						
		420						425							

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(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2522 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 310...1575

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

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GGGCTTGAG TGAGCTAAAG ATTGGGTCTT CTTGGAAATC ACCTGTCTGT TATTATTTTTT    60
AAACAATCGC TACACCTCCA AAGACTCTGC TCCTTACTCC GGTTTGGAAT GTGGCTAATG    120
ACTACCCAGT AGGGAGGATT TCTGGGGCAA ACAGCCGGAC CAGGATCCTA GTTCTCAGGC    180
ACGGAATGGC TATTGTGAGA ACAGCACCAG CAGGATCATC GCAGATCTTG GTTATGGCCA    240
CTCAGGCAAG ACGCTGTTGA GTTAAGACCC CTTTCCCATG GATGAGAAGC CACAGAAGCA    300
GTGGTATTTT ATG AGC CTC CAT TTC TTA TAC TAC TGC AGT GAA CCA ACC TTG    351
      Met Ser Leu His Phe Leu Tyr Tyr Cys Ser Glu Pro Thr Leu
        1             5             10

GAT GTG AAA ATT GCC TTT TGT CAG GGA TTC GAT AAA CAC GTG GAT GTG    399
Asp Val Lys Ile Ala Phe Cys Gln Gly Phe Asp Lys His Val Asp Val
   15             20             25             30

TCA TCT ATT GCC AAA CAT TAC AAC ATG AGC AAA AGC AAG GTG GAC AAC    447
Ser Ser Ile Ala Lys His Tyr Asn Met Ser Lys Ser Lys Val Asp Asn
           35             40             45

CAG TTC TAC AGT GTG GAA GTG GGG GAC TCA ACC TTC ACC GTT CTT AAG    495
Gln Phe Tyr Ser Val Glu Val Gly Asp Ser Thr Phe Thr Val Leu Lys
           50             55             60

CGC TAC CAG AAC CTG AAG CCA ATT GGC TCT GGG GCT CAG GGA ATA GTC    543
Arg Tyr Gln Asn Leu Lys Pro Ile Gly Ser Gly Ala Gln Gly Ile Val
           65             70             75

TGT GCT GCG TAC GAC GCT GTC CTT GAC AGA AAT GTG GCC ATT AAG AAG    591
Cys Ala Ala Tyr Asp Ala Val Leu Asp Arg Asn Val Ala Ile Lys Lys
           80             85             90

CTC AGC AGA CCC TTC CAG AAC CAA ACT CAC GCC AAG AGG GCT TAC CGG    639
Leu Ser Arg Pro Phe Gln Asn Gln Thr His Ala Lys Arg Ala Tyr Arg
           95             100            105            110

GAG CTG GTG CTC ATG AAG TGT GTG AAC CAT AAA AAC ATT ATT AGC TTA    687
Glu Leu Val Leu Met Lys Cys Val Asn His Lys Asn Ile Ile Ser Leu
          115             120             125

TTA AAT GTT TTT ACA CCC CAG AAA ACG CTG GAG GAG TTC CAA GAT GTC    735
Leu Asn Val Phe Thr Pro Gln Lys Thr Leu Glu Glu Phe Gln Asp Val
          130             135             140

TAC TTA GTG ATG GAA CTG ATG GAC GCC AAC CTG TGT CAG GTG ATT CAG    783
Tyr Leu Val Met Glu Leu Met Asp Ala Asn Leu Cys Gln Val Ile Gln
          145             150             155

ATG GAG CTG GAC CAC GAG CGG ATG TCT TAC TTG CTG TAC CAG ATG CTG    831
Met Glu Leu Asp His Glu Arg Met Ser Tyr Leu Leu Tyr Gln Met Leu
          160             165             170

TGT GGC ATC AAG CAC CTC CAC TCC GCT GGG ATC ATC CAC AGG GAC TTA    879
Cys Gly Ile Lys His Leu His Ser Ala Gly Ile Ile His Arg Asp Leu
          175             180             185             190

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AAA Lys	CCC Pro	AGT Ser	AAC Asn	ATT Ile	GTA Val	GTC Val	AAG Lys	TCT Ser	GAT Asp	TGC Cys	ACA Thr	CTG Leu	AAA Lys	ATC Ile	CTC Leu	927		
			195						200			205						
GAC Asp	TTC Phe	GGA Gly	CTG Leu	GCC Ala	AGG Arg	ACA Thr	GCG Ala	GGT Gly	ACA Thr	AGC Ser	TTC Phe	ATG Met	ATG Met	ACT Thr	CCG Pro	975		
			210						215			220						
TAT Tyr	GTG Val	GTG Val	ACG Thr	CGA Arg	TAT Tyr	TAC Tyr	AGA Arg	GCC Ala	CCT Pro	GAG Glu	GTC Val	ATC Ile	CTG Leu	GGC Gly	ATG Met	1023		
			225						230			235						
GGC Gly	TAC Tyr	AAG Lys	GAG Glu	AAC Asn	GTG Val	GAC Asp	ATA Ile	TGG Trp	TCT Ser	GTG Val	GGA Gly	TGC Cys	ATC Ile	ATG Met	GGA Gly	1071		
			240			245						250						
GAA Glu	ATG Met	GTT Val	CGC Arg	CAC His	AAA Lys	ATC Ile	CTC Leu	TTT Phe	CCC Pro	GGA Gly	AGG Arg	AGC Ser	TAT Tyr	ATT Ile	GAC Asp	1119		
255						260			265						270			
CAG Gln	TGG Trp	AAC Asn	AAA Lys	GTC Val	ATC Ile	GAG Glu	CAG Gln	CTA Leu	GGA Gly	ACT Thr	CCG Pro	TGT Cys	CCA Pro	GAG Glu	TTC Phe	1167		
			275						280						285			
ATG Met	AAG Lys	AAA Lys	TTG Leu	CAG Gln	CCC Pro	ACA Thr	GTC Val	AGA Arg	AAC Asn	TAC Tyr	GTG Val	GAG Glu	AAT Asn	CGG Arg	CCC Pro	1215		
			290						295			300						
AAG Lys	TAC Tyr	GCA Ala	GGA Gly	CTC Leu	ACC Thr	TTC Phe	CCC Pro	AAG Lys	CTC Leu	TTT Phe	CCA Pro	GAT Asp	TCC Ser	CTC Leu	TTC Phe	1263		
			305						310			315						
CCA Pro	GCG Ala	GAT Asp	TCT Ser	GAG Glu	CAC His	AAT Asn	AAA Lys	CTT Leu	AAA Lys	GCC Ala	AGC Ser	CAA Gln	GCC Ala	AGG Arg	GAT Asp	1311		
320						325						330						
TTG Leu	TTG Leu	TCT Ser	AAG Lys	ATG Met	TTA Leu	GTG Val	ATT Ile	GAC Asp	CCA Pro	GTG Val	AAG Lys	AGG Arg	ATA Ile	TCG Ser	GTG Val	1359		
335						340						345			350			
GAC Asp	GAC Asp	GCA Ala	CTG Leu	CAG Gln	CAT His	CCG Pro	TAC Tyr	ATC Ile	AAC Asn	GTT Val	TGG Trp	TAC Tyr	GAC Asp	CCG Pro	GCT Ala	1407		
			355						360			365						
GAA Glu	GTG Val	GAG Glu	GCG Ala	CCT Pro	CCG Pro	CCT Pro	CAG Gln	ATA Ile	TAT Tyr	GAT Asp	AAG Lys	CAG Gln	CTG Leu	GAT Asp	GAA Glu	1455		
			370						375			380						
AGG Arg	GAG Glu	CAC His	ACC Thr	ATC Ile	GAA Glu	GAA Glu	TGG Trp	AAA Lys	GAA Glu	CTT Leu	ATC Ile	TAC Tyr	AAG Lys	GAG Glu	GTA Val	1503		
			385						390			395						
ATG Met	AAC Asn	TCA Ser	GAA Glu	GAG Glu	AAG Lys	ACT Thr	AAG Lys	AAT Asn	GGC Gly	GTA Val	GTC Val	AAA Lys	AGC Ser	CAG Gln	CCC Pro	1551		
			400			405						410						
TCG Ser	CCT Pro	TCA Ser	GCA Ala	CAG Gln	GTG Val	CAG Gln	CAG Gln	TGAACAGCAG			TGAGAGTCTC			CCTCCATCCT			1605	
415						420												
CGGCTGTCAA			CGACATCTCC			TCCATGTCCA			CCGACCAGAC			CCTCGCATCT			GACACTGACA			1665
GCAGCCTGGA			GGCCTCGGCG			GGACCGTTGG			GTTGTTGCAG			GTGACTAGCC			GCCTGCCTGC			1725
GAAACCCAGC			GTTCTTCAGG			AGATGACGCG			ATAGAACACA			GCACACATGC			ACACACACAG			1785
CTTGCTCTCA			CACACACTCA			GCTTGCTCAC			ACACACACAC			ACACATACAC			ACAAACACAC			1845
ACTGTCTCTC			TCTCACACAC			ACACACTGTC			ACAACGCACT			CACGAAAGGT			CAAGAAAAAA			1905
ATAACAATAG			AGAGATCCAA			CATAAAATTA			AGTTAAATTT			TTCTGCGTGC			TTCTCCAAAG			1965
TTCTGTATCA			CAGCTGAGCT			GAAATGTATA			CTTAACTTCT			AGTTGCGCTC			GCTTTGGTTT			2025
CCCTCCAGCA			GTGCTTACTA			CACAAGACAA			ATCAGACACA			ATTAGAGAAA			CCTTTCCCTA			2085

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AAGTGTA	ACT	TAAGTGG	CTG	CAGAACC	CAGC	AACCTGT	AAC	TGCCCTT	CAA	ATGGCAT	GAG	2145
GAGGTGG	GCA	CGGGTCC	CGC	CCAGCAT	GTG	TGTGTCT	CTA	TCTCGCG	TCT	ACCTGCT	CTT	2205
CCGGCCT	AGT	CAGATGG	ATG	TAGATAC	AGA	TCCCGCA	TGT	GTCTGT	ATT	AAACAGC	ACT	2265
TAGAGAT	GCT	CCTGTC	AGT	TCCTCC	AGG	TCCACCA	AGA	CACACAC	CGG	GGTACC	ACAT	2325
GGTCCAT	TTT	ATGTGAT	CTA	TTACTCT	GAC	ATAAAT	CCAT	CTGTAAT	ATA	TTGCCAG	TAT	2385
ATAAGCT	GTT	TAGTTTG	TTA	ATTGCTT	AAG	CTGTAT	GTCT	TATAAG	AGAC	TATGTA	AAG	2445
GGGAAA	ATG	AGGCGT	GAAC	TCTCAG	ACCC	TTGAAG	ATGT	AGCTTCC	GAA	TTTGACC	GTT	2505
AAATGG	CACC	GTATACC										2522

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 422 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

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

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

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245					250					255					
Val	Arg	His	Lys	Ile	Leu	Phe	Pro	Gly	Arg	Ser	Tyr	Ile	Asp	Gln	Trp
260					265					270					
Asn	Lys	Val	Ile	Glu	Gln	Leu	Gly	Thr	Pro	Cys	Pro	Glu	Phe	Met	Lys
275					280					285					
Lys	Leu	Gln	Pro	Thr	Val	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr
290					295					300					
Ala	Gly	Leu	Thr	Phe	Pro	Lys	Leu	Phe	Pro	Asp	Ser	Leu	Phe	Pro	Ala
305	310					315					320				
Asp	Ser	Glu	His	Asn	Lys	Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu
325					330					335					
Ser	Lys	Met	Leu	Val	Ile	Asp	Pro	Val	Lys	Arg	Ile	Ser	Val	Asp	Asp
340					345					350					
Ala	Leu	Gln	His	Pro	Tyr	Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ala	Glu	Val
355					360					365					
Glu	Ala	Pro	Pro	Pro	Gln	Ile	Tyr	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu
370					375					380					
His	Thr	Ile	Glu	Glu	Trp	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asn
385	390					395					400				
Ser	Glu	Glu	Lys	Thr	Lys	Asn	Gly	Val	Val	Lys	Ser	Gln	Pro	Ser	Pro
405					410					415					
Ser	Ala	Gln	Val	Gln	Gln										
420															

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2347 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 - (A) NAME/KEY: Coding Sequence
 - (B) LOCATION: 615...1616

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

AGCAACTTTC	CTGACCCAGA	GGACCGGTAA	CAAGTGGCCG	GGAGCAACTT	TTGCAAATCT	60								
CTTCTGCGCC	TTAAGGCTGC	CACCGAGACT	GTAAGAAAA	GGAGAAGAGG	AACCTATACT	120								
CATACCAGTT	CGCACAGGCC	TAAGTTGGGC	GAGGCCTAGC	CGCGGCTGCC	TAGCGTCCCC	180								
CCCCCCTCA	CAGCGGAGGA	GGGGACAGTT	GTTGGAGGCC	GGGCGGCAGA	CCCGATCGCG	240								
GGCCTCCACC	GAGAATTCCG	TGACGACTGG	TCAGCACCGC	CGGAGAGCCG	CTGTTGCTGG	300								
GACTGGTCTG	CGGGCTCCAA	GGAACCGCTG	CTCCCCGAGA	GCGCTCCGTG	AGTGACCGCG	360								
ACTTTTCAAA	GCTCGGCATC	GCGCGGAGTC	CTACCAACGT	GAGTGCTAGC	GGAGTCTTAA	420								
CCCTGCGCTC	CCTGGAGCGA	ACTGGGGAGG	AGGGCTCAGG	GGGAAGCACT	GCCGTCTGGA	480								
GCGCACGCTC	TAAACAAACT	TTGTTACAGA	AGCAGGGACG	CGCGGGTATC	CCCCCGCTTC	540								
CCGGCGCGCT	GTTGCGGCCC	CGAAACTTCT	GCGCACAGCC	CAGGCTAACC	CCGCGTGAAG	600								
TGACGGACCG	TTCT	ATG	ACT	GCA	AAG	ATG	GAA	ACG	ACC	TTC	TAC	GAC	GAT	650
	Met	Thr	Ala	Lys	Met	Glu	Thr	Thr	Phe	Tyr	Asp	Asp		
	1				5						10			

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GCC	CTC	AAC	GCC	TCG	TTC	CTC	CAG	TCC	GAG	AGC	GGT	GCC	TAC	GGC	TAC	698
Ala	Leu	Asn	Ala	Ser	Phe	Leu	Gln	Ser	Glu	Ser	Gly	Ala	Tyr	Gly	Tyr	
		15					20					25				
AGT	AAC	CCT	AAG	ATC	CTA	AAA	CAG	AGC	ATG	ACC	TTG	AAC	CTG	GCC	GAC	746
Ser	Asn	Pro	Lys	Ile	Leu	Lys	Gln	Ser	Met	Thr	Leu	Asn	Leu	Ala	Asp	
	30					35					40					
CCG	GTG	GGC	AGT	CTG	AAG	CCG	CAC	CTC	CGC	GCC	AAG	AAC	TCG	GAC	CTT	794
Pro	Val	Gly	Ser	Leu	Lys	Pro	His	Leu	Arg	Ala	Lys	Asn	Ser	Asp	Leu	
	45				50				55						60	
CTC	ACG	TCG	CCC	GAC	GTC	GGG	CTG	CTC	AAG	CTG	GCG	TCG	CCG	GAG	CTG	842
Leu	Thr	Ser	Pro	Asp	Val	Gly	Leu	Leu	Lys	Leu	Ala	Ser	Pro	Glu	Leu	
				65					70					75		
GAG	CGC	CTG	ATC	ATC	CAG	TCC	AGC	AAT	GGG	CAC	ATC	ACC	ACT	ACA	CCG	890
Glu	Arg	Leu	Ile	Ile	Gln	Ser	Ser	Asn	Gly	His	Ile	Thr	Thr	Thr	Pro	
			80					85					90			
ACC	CCC	ACC	CAG	TTC	TTG	TGC	CCC	AAG	AAC	GTG	ACC	GAC	GAG	CAG	GAG	938
Thr	Pro	Thr	Gln	Phe	Leu	Cys	Pro	Lys	Asn	Val	Thr	Asp	Glu	Gln	Glu	
		95					100					105				
GGC	TTC	GCC	GAG	GGC	TTC	GTG	CGC	GCC	CTG	GCT	GAA	CTG	CAT	AGC	CAG	986
Gly	Phe	Ala	Glu	Gly	Phe	Val	Arg	Ala	Leu	Ala	Glu	Leu	His	Ser	Gln	
	110					115					120					
AAC	ACG	CTT	CCC	AGT	GTC	ACC	TCC	GCG	GCA	CAG	CCG	GTC	AGC	GGG	GCG	1034
Asn	Thr	Leu	Pro	Ser	Val	Thr	Ser	Ala	Ala	Gln	Pro	Val	Ser	Gly	Ala	
	125				130					135					140	
GGC	ATG	GTG	GCT	CCC	GCG	GTG	GCC	TCA	GTA	GCA	GGC	GCT	GGC	GGC	GGT	1082
Gly	Met	Val	Ala	Pro	Ala	Val	Ala	Ser	Val	Ala	Gly	Ala	Gly	Gly	Gly	
				145					150					155		
GGT	GGC	TAC	AGC	GCC	AGC	CTG	CAC	AGT	GAG	CCT	CCG	GTC	TAC	GCC	AAC	1130
Gly	Gly	Tyr	Ser	Ala	Ser	Leu	His	Ser	Glu	Pro	Pro	Val	Tyr	Ala	Asn	
			160					165					170			
CTC	AGC	AAC	TTC	AAC	CCG	GGT	GCG	CTG	AGC	AGC	GGC	GGT	GGG	GCG	CCC	1178
Leu	Ser	Asn	Phe	Asn	Pro	Gly	Ala	Leu	Ser	Ser	Gly	Gly	Gly	Ala	Pro	
		175					180					185				
TCC	TAT	GGC	GCG	GCC	GGG	CTG	GCC	TTT	CCC	TCG	CAG	CCG	CAG	CAG	CAG	1226
Ser	Tyr	Gly	Ala	Ala	Gly	Leu	Ala	Phe	Pro	Ser	Gln	Pro	Gln	Gln	Gln	
	190					195					200					
CAG	CAG	CCG	CCT	CAG	CCG	CCG	CAC	CAC	TTG	CCC	CAA	CAG	ATC	CCG	GTG	1274
Gln	Gln	Pro	Pro	Gln	Pro	Pro	His	His	Leu	Pro	Gln	Gln	Ile	Pro	Val	
	205				210					215					220	
CAG	CAC	CCG	CGG	CTG	CAA	GCC	CTG	AAG	GAA	GAG	CCG	CAG	ACC	GTG	CCG	1322
Gln	His	Pro	Arg	Leu	Gln	Ala	Leu	Lys	Glu	Glu	Pro	Gln	Thr	Val	Pro	
				225					230					235		
GAG	ATG	CCG	GGA	GAG	ACG	CCG	CCC	CTG	TCC	CCT	ATC	GAC	ATG	GAG	TCT	1370
Glu	Met	Pro	Gly	Glu	Thr	Pro	Pro	Leu	Ser	Pro	Ile	Asp	Met	Glu	Ser	
		240						245					250			
CAG	GAG	CGG	ATC	AAG	GCA	GAG	AGG	AAG	CGC	ATG	AGG	AAC	CGC	ATT	GCC	1418
Gln	Glu	Arg	Ile	Lys	Ala	Glu	Arg	Lys	Arg	Met	Arg	Asn	Arg	Ile	Ala	
		255					260					265				
GCC	TCC	AAG	TGC	CGG	AAA	AGG	AAG	CTG	GAG	CGG	ATC	GCT	CGG	CTA	GAG	1466
Ala	Ser	Lys	Cys	Arg	Lys	Arg	Lys	Leu	Glu	Arg	Ile	Ala	Arg	Leu	Glu	
	270					275					280					
GAA	AAA	GTG	AAA	ACC	TTG	AAA	GCG	CAA	AAC	TCC	GAG	CTG	GCA	TCC	ACG	1514
Glu	Lys	Val	Lys	Thr	Leu	Lys	Ala	Gln	Asn	Ser	Glu	Leu	Ala	Ser	Thr	
	285				290					295					300	
GCC	AAC	ATG	CTC	AGG	GAA	CAG	GTG	GCA	CAG	CTT	AAG	CAG	AAA	GTC	ATG	1562
Ala	Asn	Met	Leu	Arg	Glu	Gln	Val	Ala	Gln	Leu	Lys	Gln	Lys	Val	Met	
				305					310					315		

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AAC CAC GTT AAC AGT GGG TGC CAA CTC ATG CTA ACG CAG CAG TTG CAA	1610
Asn His Val Asn Ser Gly Cys Gln Leu Met Leu Thr Gln Gln Leu Gln	
320 325 330	
ACG TTT TGAGAACAGA CTGTCAGGGC TGAGGGGCAA TGGAAGAAAA AAAATAACAG	1666
Thr Phe	
AGACAAACTT GAGAACTTGA CTGGTTGCGA CAGAGAAAAA AAAAGTGTCC GAGTACTGAA	1726
GCCAAGGGTA CACAAGATGG ACTGGGTTTCG GACTGACGGC GCCCCCAGTG TGCTCTGGAG	1786
TGGGAAGGAC GTGGCGCGCC TGGCTTTGGC GTGGAGCCAG AGAGCAGGCC TATTGGCCCCG	1846
CAGACTTTGC GGAGCGCTGT GCCGCGCGCG ACCAGAACGA TGGACTTTTC GTTAACATTG	1906
ACCAAGAACT GCATGGACCT AACATTCGAT CTCATTTCAGT ATTAAAGGGG GGTGGGAGGG	1966
GTTACAAACT GCAATAGAGA CTGTAGATTG CTTCTGTAGT GTCCTTAAC ACAAAGCAGG	2026
GAGGGCTGGG AAGGGGGGGA GGCTTGTAAG TGCCAGGCTA GACTGCAGAT GAACTCCCCT	2086
GGCCTGCCTC TCTCAACTGT GTATGTACAT ATATATTTTT TTTTAATTG ATGAAAGCTG	2146
ATTACTGTCA ATAAACAGCT TCCTGCCTTT GTAAGTTATT CCATGTTTGT TTGTTTGGGT	2206
GTCCTGCCCA GTGTTTGTAA ATAAGAGATT TGAAGCATTC TGAGTTTACC ATTTGTAATA	2266
AAGTATATAA TTTTTTTATG TTTTGTCTTCT GAAAATTTCC AGAAAGGATA TTTAAGAAAA	2326
TACAATAAAC TATTGAAAAG T	2347

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 334 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

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

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

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165					170					175						
Asn	Pro	Gly	Ala	Leu	Ser	Ser	Gly	Gly	Gly	Ala	Pro	Ser	Tyr	Gly	Ala	
180					185					190						
Ala	Gly	Leu	Ala	Phe	Pro	Ser	Gln	Pro	Gln	Gln	Gln	Gln	Gln	Pro	Pro	
195					200					205						
Gln	Pro	Pro	His	His	Leu	Pro	Gln	Gln	Ile	Pro	Val	Gln	His	Pro	Arg	
210					215					220						
Leu	Gln	Ala	Leu	Lys	Glu	Glu	Pro	Gln	Thr	Val	Pro	Glu	Met	Pro	Gly	
225					230					235					240	
Glu	Thr	Pro	Pro	Leu	Ser	Pro	Ile	Asp	Met	Glu	Ser	Gln	Glu	Arg	Ile	
245					250					255						
Lys	Ala	Glu	Arg	Lys	Arg	Met	Arg	Asn	Arg	Ile	Ala	Ala	Ser	Lys	Cys	
260					265					270						
Arg	Lys	Arg	Lys	Leu	Glu	Arg	Ile	Ala	Arg	Leu	Glu	Glu	Lys	Val	Lys	
275					280					285						
Thr	Leu	Lys	Ala	Gln	Asn	Ser	Glu	Leu	Ala	Ser	Thr	Ala	Asn	Met	Leu	
290					295					300						
Arg	Glu	Gln	Val	Ala	Gln	Leu	Lys	Gln	Lys	Val	Met	Asn	His	Val	Asn	
305					310					315					320	
Ser	Gly	Cys	Gln	Leu	Met	Leu	Thr	Gln	Gln	Leu	Gln	Thr	Phe			
325					330											

(2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3967 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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

ATACCAGAGA CTCAAAAAAA AAAAAAAGT TCCAGATTGC TGGACAATGA CCCGGGTCTC	60
ATCCCTTGAC CCTGGGAACC GGGTCCACAT TGAATCAGGT GCGAATGTTC GCTCGCCTTC	120
TCTGCCTTTC CCGCCTCCCC TCCCCCGGCC GCGGCCCGG TTCCCCCCTT GCGCTGCACC	180
CTCAGAGTTG GCTGCAGCCG GCGAGCTGTT CCCGTCAATC CCTCCCTCCT TTACACAGGA	240
TGTCCATATT AGGACATCTG CGTCAGCAGG TTTCCACGGC CGGTCCCTGT TGTTCTGGGG	300
GGGGGACCAT CTCCGAAATC CTACACGCGG AAGGTCTAGG AGACCCCTA AGATCCCAA	360
TGTGAACACT CATAGGTGAA AGATGTATGC CAAGACGGGG GTTGAAAGCC TGGGGCGTAG	420
AGTTGACGAC AGAGCGCCCG CAGAGGGCCT TGGGGCGCGC TTCCCCCCCC TTCCAGTTCC	480
GCCCAGTGAC GTAGGAAGTC CATCCATTCA CAGCGCTTCT ATAAAGGCGC CAGCTGAGGC	540
GCCTACTACT CCAACGCGA CTGCAGCGAG CAACTGAGAA GACTGGATAG AGCCGGCGGT	600
TCCGCGAACG AGCAGTGACC GCGCTCCCAC CCAGCTCTGC TCTGCAGCTC CCACCAGTGT	660
CTACCCCTGG ACCCCTTGCC GGGCTTTCCC CAACTTCGA CCATGATGTT CTCGGGTTTC	720
AACGCCGACT ACGAGGCGTC ATCCTCCCGC TGCACTAGCG CCTCCCCGGC CGGGGACAGC	780
CTTTCCTACT ACCATTCCCC AGCCGACTCC TTCTCCAGCA TGGGCTCTCC TGTCAACAGC	840
CAGGTGAGTT TGGCTTTGTG TAGCCGCCAG GTCCGCGCTG AGGGTCGCCG TGGAGGAGAC	900
ACTGGGGTGT GACTCGCAGG GCGGGGGGG TCTTCCTTTT TCGCTCTGGA GGGAGACTGG	960

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CGCGGTCAGA	GCAGCCTTAG	CCTGGGAACC	CAGGACTTGT	CTGAGCGCGT	GCACACTTGT	1020
CATAGTAAGA	CTTAGTGACC	CCTTCCCGCG	CGGCAGGTTT	ATTCTGAGTG	GCCTGCCTGC	1080
ATTCTTCTCT	CGGCCGACTT	GTTTCTGAGA	TCAGCCGGGG	CCAACAAGTC	TCGAGCAAAG	1140
AGTCGCTAAC	TAGAGTTTGG	GAGGCGGCAA	ACCGCGGCAA	TCCCCCTCC	CGGGGCAGCC	1200
TGGAGCAGGG	AGGAGGGAGG	AGGGAGGAGG	GTGCTGCGGG	CGGGTGTGTA	AGGCAGTTTC	1260
ATTGATAAAA	AGCGAGTTCA	TTCTGGAGAC	TCCGGAGCAG	CGCCTGCGTC	AGCGCAGACG	1320
TCAGGGATAT	TTATAACAAA	CCCCCTTTCG	AGCGAGTGAT	GCCGAAGGGA	TAACGGGAAC	1380
GCAGCAGTAG	GATGGAGGAG	AAAGGCTGCG	CTGCGGAATT	CAAGGGAGGA	TATTGGGAGA	1440
GCTTTTATCT	CCGATGAGGT	GCATACAGGA	AGACATAAGC	AGTCTCTGAC	CGGAATCTTG	1500
CTCTCTCCCT	GCTTCATGCG	ACACTAGGGC	CACTTGCTCC	ACCTGTGTCT	GGAACCTCCT	1560
CGCTCACCTC	CGCTTTCCTC	TTTTTGTTTT	GTTTCAGGAC	TTTTGCGCAG	ATCTGTCCGT	1620
CTCTAGTGCC	AACTTTATCC	CCACGGTGAC	AGCCATCTCC	ACCAGCCCAG	ACCTGCAGTG	1680
GCTGGTGACG	CCCACTCTGG	TCTCCTCCGT	GGCCCCATCG	CAGACCAGAG	CGCCCCATCC	1740
TTACGGACTC	CCCACCCAGT	CTGCTGGGGC	TTACGCCAGA	GCGGGAATGG	TGAAGACCGT	1800
GTCAGGAGGC	AGAGCGCAGA	GCATCGGCAG	AAGGGGCAAA	GTAGAGCAGG	TGAGCAGCGA	1860
TTCTGGACCT	TTGTGGGCTG	GGGGGGGGGG	GGGGGGCGGA	GACTGACGCA	CAGACCACAC	1920
AACAGAGAAG	GGACGCTACT	GACTGCACTT	CCTGACCAGG	AGCTGTGGCT	GCTAGCCCTT	1980
TCCCTCCCTT	GTCAGATTTT	GACAGTTGGA	CCCAAGACAA	ACTCTAGACA	GTTTCCCTGA	2040
CAGCTTCCTA	CTTCATTCTC	TAGCCGGGGA	GCTTCTTTGT	TCCCTGCTA	AAGATCTCAC	2100
TTTAAATGCA	AATCACACTC	TGCCTGCCAA	CTGCAGGTTA	GAAAACTGC	TTCACCGAGA	2160
GGTGCGGGTG	CTGTAGGAGC	CAGTTTCACT	GGGGTGACTG	AATGGAGGTG	ACACTAGACA	2220
ACCTTAACTG	AATGTTGGTC	CTTTTCTTCT	ATAGCTATCT	CCTGAAGAGG	AAGAGAAAGG	2280
GAGAATCCGA	AGGGAACGGA	ATAAGATGGC	TGCAGCCAAG	TGCCGGAATC	GGAGGAGGGA	2340
GCTGACAGAT	ACACTCCAAG	CGGTAGGTTG	AACCAGCTGC	TGCTCCTGAA	ACTTTATTAA	2400
AGTTGGAGCT	TGGGACTATG	GGCGCAGGGT	CCTTGAGCAT	GCCCGTGTCT	TATGCTTTCT	2460
TATATCTCTC	CCTATGCAGG	AGACAGATCA	ACTTGAAGAT	GAGAAGTCTG	CGTTGCAGAC	2520
TGAGATTGCC	AATCTGCTGA	AAGAGAAGGA	AAAAGTGGAG	TTTATTTTGG	CAGCCCACCG	2580
ACCTGCCTGC	AAGATCCCCG	ATGACCTTGG	CTTCCCAGAG	GAGATGTCTG	TGGCCTCCCT	2640
GGATTTGACT	GGAGGTCTGC	CTGAGGCTTC	CACCCCAGAG	TCTGAGGAGG	CCTTCACCCT	2700
GCCCCTTCTC	AACGACCCTG	AGCCCAAGCC	ATCCTTGAGG	CCAGTCAAGA	GCATCAGCAA	2760
CGTGAGAGCT	AAGGCAGAAC	CCTTTGATGA	CTTCTTGTTT	CCGGCATCAT	CTAGGCCCAG	2820
TGGCTCAGAG	ACCTCCCGCT	CTGTGCCAGA	TGTGGACCTG	TCCGGTTCCT	TCTATGCAGC	2880
AGACTGGGAG	CCTCTGCACA	GCAATTCCTT	GGGGATGGGG	CCCATGGTCA	CAGAGCTGGA	2940
GCCCCGTGTG	ACTCCCGTGG	TCACCTGTAC	TCCGGGCTGC	ACTACTTACA	CGTCTTCCTT	3000
TGTCTTCACC	TACCCTGAAG	CTGACTCCTT	CCCAAGCTGT	GCCGCTGCCC	ACCGAAAGGG	3060
CAGCAGCAGC	AACGAGCCCT	CCTCCGACTC	CCTGAGCTCA	CCCACGCTGC	TGGCCCTGTG	3120
AGCAGTCAGA	GAAGGCAAGG	CAGCCGGCAT	CCAGACGTGC	CACTGCCCCG	GCTGGTGCAT	3180
TACAGAGAGG	AGAAACACGT	CTTCCCTCGA	AGGTTCCCGT	CGACCTAGGG	AGGACCTTAC	3240

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CTGTTCGTGA AACACACCAG GCTGTGGGCC TCAAGGACTT GCAAGCATCC ACATCTGGCC	3300
TCCAGTCCTC ACCTCTTCCA GAGATGTAGC AAAAACAAAA CAAACAAAA CAAAAAACCG	3360
CATGGAGTGT GTTGTTCCCTA GTGACACCTG AGAGCTGGTA GTTAGTAGAG CATGTGAGTC	3420
AAGGCCTGGT CTGTGTCTCT TTTCTCTTTC TCCTTAGTTT TCTCATAGCA CTAACATAATC	3480
TGTTGGGTTC ATTATTGGAA TTAACCTGGT GCTGGATTGT ATCTAGTGCA GCTGATTTTA	3540
ACAATACCTA CTGTGTTCCCT GGCAATAGCG TGTTCCAATT AGAAACGACC AATATTAAAC	3600
TAAGAAAAGA TAGGACTTTA TTTTCCAGTA GATAGAAATC AATAGCTATA TCCATGTACT	3660
GTAGTCCTTC AGCGTCAATG TTCATTGTCA TGTTACTGAT CATGCATTGT CGAGGTGGTC	3720
TGAATGTTCT GACATTAACA GTTTTCCATG AAAACGTTTT TATTGTGTTT TCAATTTATT	3780
TATTAAGATG GATTCTCAGA TATTTATATT TTTATTTTAT TTTTTTCTAC CCTGAGGTCT	3840
TTCGACATGT GGAAAGTGAA TTTGAATGAA AAATTTTAAG CATTGTTTGC TTATTGTTCC	3900
AGGACATTGT CAATAAAAGC ATTTAAGTTG AATGCGACCA CCTTCTTGCT CTCTTTATTC	3960
TCAGTTT	3967

(2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 380 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

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

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195					200					205					
Asp	Leu	Gly	Phe	Pro	Glu	Glu	Met	Ser	Val	Ala	Ser	Leu	Asp	Leu	Thr
210						215					220				
Gly	Gly	Leu	Pro	Glu	Ala	Ser	Thr	Pro	Glu	Ser	Glu	Glu	Ala	Phe	Thr
225					230					235					240
Leu	Pro	Leu	Leu	Asn	Asp	Pro	Glu	Pro	Lys	Pro	Ser	Leu	Glu	Pro	Val
				245					250					255	
Lys	Ser	Ile	Ser	Asn	Val	Glu	Leu	Lys	Ala	Glu	Pro	Phe	Asp	Asp	Phe
			260					265					270		
Leu	Phe	Pro	Ala	Ser	Ser	Arg	Pro	Ser	Gly	Ser	Glu	Thr	Ser	Arg	Ser
		275					280					285			
Val	Pro	Asp	Val	Asp	Leu	Ser	Gly	Ser	Phe	Tyr	Ala	Ala	Asp	Trp	Glu
290						295					300				
Pro	Leu	His	Ser	Asn	Ser	Leu	Gly	Met	Gly	Pro	Met	Val	Thr	Glu	Leu
305					310					315					320
Glu	Pro	Leu	Cys	Thr	Pro	Val	Val	Thr	Cys	Thr	Pro	Gly	Cys	Thr	Thr
				325					330					335	
Tyr	Thr	Ser	Ser	Phe	Val	Phe	Thr	Tyr	Pro	Glu	Ala	Asp	Ser	Phe	Pro
			340					345					350		
Ser	Cys	Ala	Ala	Ala	His	Arg	Lys	Gly	Ser	Ser	Ser	Asn	Glu	Pro	Ser
		355					360					365			
Ser	Asp	Ser	Leu	Ser	Ser	Pro	Thr	Leu	Leu	Ala	Leu				
370						375					380				

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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

AAGAAATGGA GGCTCATAAA TACCACAGCT 30

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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

ATTGGAAGAA GACCAAAGCA AGAGCAACTA 30

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:
TAAGTAAGTA GTGCTGTATG AATACAGACA 30

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:
TACTGGCAAT ATATTACAGA TGGGTTTATG 30

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
GTGTGCAGCT TATGATGCTA TTCTTGAA 28

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:
CGCGTCACCA CATACGGAGT CATC 24

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:
CTGGAGGAGT TCCAAGATGT CTACT 25

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

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

TGGAAAGAGC TTGGGGAAGG TGAG

24

What is claimed is:

1-17. (canceled)**18.** A method for identifying a modulator of c-Jun N-terminal kinase (JNK3), the method comprising:

(a) exposing a cell to a stress signal in the presence of a test compound under conditions that allow the cell to phosphorylate c-Jun; and

(b) determining the level of phosphorylated c-Jun in the cell,

wherein a difference in the level of phosphorylated c-Jun determined in (b) compared to the level of phosphorylated c-Jun in the absence of the test compound indicates that the test compound is a modulator of JNK3.

19. The method of claim 18, wherein the cell is a neuronal cell.**20.** The method of claim 19, wherein the neuronal cell is a hippocampal cell.**21.** The method of claim 18, wherein the test compound is an antisense nucleic acid, an oligonucleotide, a ribozyme, a peptide, a peptidomimetic, an amino acid, a phosphopeptide, a small organic molecule, or a small inorganic molecule.**22.** A method for identifying a modulator of c-Jun N-terminal kinase (JNK3), the method comprising:

(a) exposing a cell to a stress signal in the presence of a test compound under conditions that allow the cell to increase AP-1 transcription activity; and

(b) determining the level of AP-1 transcription activity in the cell,

wherein a difference in the level of AP-1 transcription activity determined in (b) compared to the level of AP-1 transcription activity in the absence of the test compound indicates that the test compound is a modulator of JNK3.

23. The method of claim 22, wherein the cell is a neuronal cell.**24.** The method of claim 23, wherein the neuronal cell is a hippocampal cell.**25.** The method of claim 22, wherein AP-1 transcription activity is measured using a transgene comprising an AP-1 binding site and a reporter gene.**26.** The method of claim 22, wherein the test compound is an antisense nucleic acid, an oligonucleotide, a ribozyme, a peptide, a peptidomimetic, an amino acid, a phosphopeptide, a small organic molecule, or a small inorganic molecule.**27.** A method of identifying a compound that modulates excitotoxicity, the method comprising:

(a) identifying a modulator of c-Jun N-terminal kinase (JNK3) expression;

(b) incubating a cell that can exhibit excitotoxicity with the modulator of JNK3 expression under conditions

sufficient for the cell to exhibit excitotoxicity in the absence of the modulator of JNK3 expression; and

(c) determining excitotoxicity in the cell after (b),

wherein a difference in the level of excitotoxicity determined in (c) compared to the level of excitotoxicity in the absence of the modulator of JNK3 expression indicates that the modulator of JNK3 expression modulates excitotoxicity.

28. The method of claim 27, wherein the modulator is an antisense nucleic acid, an oligonucleotide, a ribozyme, a peptide, a peptidomimetic, an amino acid, a phosphopeptide, a small organic molecule, or a small inorganic molecule.**29.** A method of identifying a compound that modulates excitotoxicity, the method comprising:

(a) identifying a modulator of c-Jun N-terminal kinase (JNK3) activity;

(b) incubating a cell that can exhibit excitotoxicity with the modulator of JNK3 activity under conditions for the cell to exhibit excitotoxicity in the absence of the modulator of JNK3 activity; and

(c) determining excitotoxicity in the cell after (b),

wherein a difference in the level of excitotoxicity determined in (c) compared to the level of excitotoxicity in the absence of the modulator of JNK3 activity indicates that the modulator of JNK3 activity modulates excitotoxicity.

30. The method of claim 29, wherein the modulator is an oligonucleotide, a peptide, a peptidomimetic, an amino acid, a phosphopeptide, a small organic molecule, or a small inorganic molecule.**31.** A method of identifying a compound that modulates excitotoxicity, the method comprising:

(a) identifying a modulator of c-Jun N-terminal kinase (JNK3) substrate binding;

(b) incubating a cell that can exhibit excitotoxicity with the modulator of JNK3 substrate binding under conditions for the cell to exhibit excitotoxicity in the absence of the modulator of JNK3 substrate binding; and

(c) determining excitotoxicity in the cell after (b),

wherein a difference in the level of excitotoxicity determined in (c) compared to the level of excitotoxicity in the absence of the modulator of JNK3 substrate binding indicates that the modulator of JNK3 substrate binding modulates excitotoxicity.

32. The method of claim 31, wherein the modulator is an oligonucleotide, a peptide, a peptidomimetic, an amino acid, a phosphopeptide, a small organic molecule, or a small inorganic molecule.

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