INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) Title: ROBO: A FAMILY OF POLYPEPTIDES AND NUCLEIC ACIDS INVOLVED IN NERVE GUIDANCE
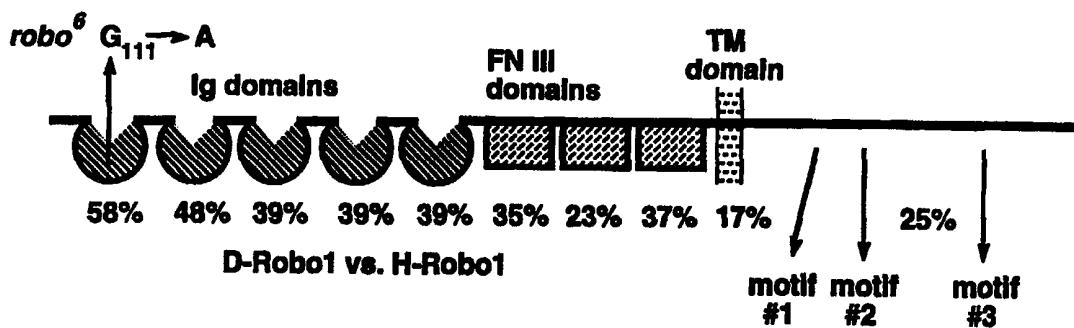
(57) Abstract

Robo1 and Robo2 polypeptides may be produced recombinantly from transformed host cells from the disclosed Robo encoding nucleic acids or purified from human cells. The invention provides isolated Robo hybridization probes and primers capable of specifically hybridizing with the disclosed Robo genes, Robo–specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

ROBO: A FAMILY OF POLYPEPTIDES AND NUCLEIC ACIDS INVOLVED IN NERVE GUIDANCE

Inventors: Corey S. Goodman, Thomas Kidd, Kevin J. Mitchell and Guy Tear

This application claims priority to US Provisional Application No. 60/062921 filed Oct 20, 1997 by Corey S. Goodman, Thomas Kidd, Kevin J. Mitchell, and Guy Tear and entitled *Robo: A Novel Family of Genes and Proteins*.

INTRODUCTION

Field of the Invention

The field of this invention is proteins involved in nerve cell guidance.

Background

Bilaterally symmetric nervous systems, such as those found in insects and vertebrates, have special midline structures that establish a partition between the two mirror image halves. Axons that link the two sides of the nervous system project toward and across the midline, forming axon commissures. These commissural axons project toward the midline, at least in part, by responding to long-range chemoattractants emanating from the midline. One important class of midline chemoattractants are the netrins (Serafini et al., 1994; Kennedy et al., 1994), guidance signals whose structure, function, and midline expression is evolutionarily conserved from nematodes and fruit flies to vertebrates (Hedgecock et al., 1990; Wadsworth et al., 1996; Mitchell et al., 1996; Harris et al., 1996). The attractive actions of netrins appear to be mediated by growth cone receptors of the DCC subfamily of the immunoglobulin (Ig) superfamily (Keino-Masu et al., 1996; Chan et al., 1996; Kolodziej et al., 1996).

The midline also provides important short-range guidance signals. This is best illustrated by considering the different classes of axon projections in the spinal cord of vertebrates or the nerve cord of insects. Although some growth cones extend away from the midline, most extend towards or along the midline during some segment of their trajectory. Certain classes of growth cones either extend towards the midline or longitudinally along it

and yet never cross it. Most growth cones (~90% in the Drosophila CNS), however, do cross the midline. After crossing, the majority of these growth cones turn to project longitudinally, growing along or near the midline. Interestingly, these axons never cross the midline again, despite navigating in the vicinity of other axons that continue to cross.

What midline signals and growth cone receptors control whether growth cones do or do not cross the midline? After crossing once, what mechanism prevents these growth cones from crossing again? Studies in the chick (Stoeckli and Landmesser, 1995; Stoeckli et al., 1997) and grasshopper (Myers and Bastiani, 1993) embryos have led to the suggestion that the midline contains a contact-mediated repellent, and that commissural growth cones must overcome this repellent to cross the midline. For example, this notion that the midline can be repulsive even to growth cones that cross it is supported by time-lapse imaging of the first commissural growth cone in the grasshopper embryo. On contacting the midline, this growth cone often abruptly retracts, although ultimately it overcomes the repulsion and crosses the midline.

One approach to find the genes encoding the components of such a midline guidance system is to screen for mutations in which either too many or too few axons cross the midline. Such a large-scale mutant screen was previously conducted in Drosophila and led to the identification of two key mutations: *commissureless* (*comm*) and *roundabout* (*robo*) (Seeger et al., 1993; reviewed by Tear et al., 1993). In *comm* mutant embryos, commissural growth cones initially orient toward the midline but then fail to cross it and instead recoil and extend on their own side. *comm* encodes a novel surface protein expressed on midline cells. As commissural growth cones contact and traverse the CNS midline, Comm protein is apparently transferred from midline cells to commissural axons (Tear et al., 1996). In *robo* mutant embryos, many growth cones that normally extend only on their own side instead now project across the midline, and axons that normally cross the midline only once instead appear to cross and recross multiple times (Seeger et al, 1993; Kidd et al., 1997). Double mutants of *comm* and *robo* display a *robo*-like phenotype.

Here we disclose the characterization of *robo* across animal species. *robo* encodes a new class of guidance receptor with 5 Ig domains, 3 fibronectin (FN) type III domains, a transmembrane domain, and a long cytoplasmic domain. Robo defines a new subfamily of Ig superfamily proteins that is highly conserved from fruit flies to mammals. The results of protein expression and transgenic rescue experiments indicate that Robo functions as the

2

gatekeeper controlling midline crossing and that Robo responds to an unknown midline repellent.

SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to Robo1 and Robo2, collectively Robo) polypeptides, related nucleic acids, polypeptide domains thereof having Robo-specific structure and activity, and modulators of Robo function. Robo polypeptides can regulate cell, especially nerve cell, function and morphology. The polypeptides may be produced recombinantly from transformed host cells from the subject Robo polypeptide encoding nucleic acids or purified from mammalian cells. The invention provides isolated Robo hybridization probes and primers capable of specifically hybridizing with natural Robo genes, Robo-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g. genetic hybridization screens for Robo transcripts), therapy (e.g. Robo inhibitors to promote nerve cell growth) and in the biopharmaceutical industry (e.g. as immunogens, reagents for isolating Robo genes and polypeptides, reagents for screening chemical libraries for lead pharmacological agents, etc.).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Organization of the roundabout Genomic Locus

(A) Cosmid chromosome walk through the 58F/59A region of the 2nd chromosome. The position of deficiency breakpoints within the cosmids used are shown in the top two rows. Identified transcripts from the walk are shown below the cosmids. The 12-1 transcript corresponds to the *robo* gene; the direction of transcription is distal to proximal. The location of the 16kb XbaI genomic rescue fragment is indicated below.

(B) Position and size of introns within the *robo* transcript. Coding sequence is indicated by the thicker part of the line. Introns are represented by gaps. The transcript is shown 3'-5' to reflect its orientation in (A).

Figure 2 Structure of Robo Protein

Schematic of the structure of Drosophila Robo protein. The position of the Immunoglobulin (Ig), fibronectin (FN) and transmembrane (TM) domains and the amino acid substitution in *robo[6]* are shown. Percent amino acid identity between Drosophila Robo 1 and Human Robo 1

3

is indicated for each domain.

DETAILED DESCRIPTION OF THE INVENTION

The nucleotide sequences of exemplary natural cDNAs encoding drosophila 1, drosophila 2, C. elegans, human 1, human 2 and mouse 1 Robo polypeptides are shown as SEQ ID NOS:1, 3, 5, 7, 9 and 11, respectively, and the full conceptual translates are shown as SEQ ID NOS:2, 4, 6, 8, 10 and 12. The Robo polypeptides of the invention include incomplete translates of SEQ ID NOS:1, 3, 5, 7, 9 and 11 and deletion mutants of SEQ ID NOS:2, 4, 6, 8, 10 and 12, which translates and deletion mutants have Robo-specific amino acid sequence, binding specificity or function. Preferred translates/deletion mutants comprise at least a 6, preferably at least an 8, more preferably at least a 32, most preferably at least a 64 residue domain of the translates. In a particular embodiment, the deletion mutants comprise one or more structural/functional Robo immunoglobulin, fibronectin or cytoplasmic motif domains described herein. For example, soluble forms of the disclosed Robo polypeptides which comprise one or more Robo IG domains, and especially fusions of two or more Robo IG domains, particularly fusions of IG#1 and #2, provide competitive inhibitors of Robo-mediated signaling. Exemplary such deletion mutants and recombined deletion mutant fusions include human Robo 1 (SEQ ID NO:8) residues 1-67; 68-167; 168-259; 260-350; 351-451; 1-167; 1-259; 1-350; 1-451; 68-259; 1-67 joined to 168-259; and 1-67 joined to 260-451.

Other deletion mutants provide Robo-specific antigens and/or immunogens, especially when coupled to carrier proteins as described below. Generic Robo-specific peptides are readily apparent as conserved regions in the aligned Robo polypeptide sequences of Table 1.

Table 1. Sequence Alignment of Robo Family Members: The complete amino acid alignment of the predicted Robo proteins encoded by *drosophila robo 1* (D1, SEQ ID NO:2) and Human *robo 1* (H1, SEQ ID NO:8) are shown. The extracellular domain of *C.elegans robo* (CE, SEQ ID NO:6; Sax-3; Zallen et al., 1997), the extracellular domain of *Drosophila robo 2* (D2, SEQ ID NO:4), and partial sequence of Human *robo 2* (H2, SEQ ID NO:10) are also aligned. The D2 sequence was predicted by the gene-finder program Grail. The position of immunoglobulin domains (Ig), fibronectin domains (FN), the transmembrane domain (TM), and conserved cytoplasmic motifs are indicated. The extracellular domain of rat *robo 1* is nearly identical to H1.

```
mH............PMHpENHAIaRSTSTTNNPSrsRSSRMWLlpAWLLLVLVASNGLP    47  D1
m.FNRKTLlCTi.llVlQA..............vIrsFCEDASNlA...............  30  CE
mKWKHVPFlVMiSllSlSpNHLFLaQLIPDPEDvErG.NDHGTPIpTSDNDDNSLGYTGS   59  H1
```

```
        >IG #1
AVrGQYQSpriiehpTdlvvKknepatlnckVegKpEptiewfkdgepvStn..EKKshr  105  D1
        GENpriiehpMdTTvPknDpFtFncQaegNptptiQwfkdgRELKt...dTGshr        D2
........pViiehpIdVvvsRgSpatlncGaK.PStAKiTwykdgQpvItnkEQVNshr   81  CE
RLrQEDFPpriVehpSdlIvskgepatlnckaegRptptiewykGgeRvEtDkDdPRshr  119  H1
```

```
                                                        >IG #2
VQFKDgAlffYriMQgkkeQ..dGgEywcvaknRVgQavsrHaslqIavlrddfrvepKd  163  D1
iMlpAgGlfflkvIhSrReS..dagTywcEakneFgVaRsrnaTlqvavlrdEfrLepAN       D2
iVlDTgslfLlkvNSgkNGKDSdagAyYcvaSneHgeVKsNEGslKLaMlrEdfrvRpRT  141  CE
MLlpSgslfflriVhgrkSRP.dEgVyVcvaRnYLgeavsHnaslEvaIlrddfrQNpSd  178  H1
```

```
trvaKgeTallecgppKgIpeptLIwIkdgVplddLKAmSFGASSrVrivdggnlLiSNv  223  D1
trvaQgeValmecgAprgSpepQiswrkNgQTlNL......VGNKririvdggnlAiQEA       D2
vQALGgeMavlecSpprgFpepVVswrkdDKElRI.QDmP.....rYTLHSDgnlIiDPv  195  CE
vMvaVgePavmecQpprgHpeptiswKkdgSpldd.......KDEri.TIRggKlMiTYT  230  H1
```

```
                                    >IG #3
EPIdEgNyKcIaQnLvgtresSYaKlIvQvkpYfMkepkdqVMLYgQTaTfHcSvggdpP  283  D1
rQsdDgRyqcvVKnVvgtresATaFlKvHvrpFLIRGpQnqtAVvgSsvVfQcrIggdpL       D2
DRsdSgTyqcvaNnmvgerVsNPaRlSvFekpKfEQepkdMtvDvgAAvLfDcrvTgdpQ  255  CE
rKsdAgKyVcvGTnmvgeresEVaElTvLerpSfVkRpSnLAvTvDDsaEfKcEARgdpV  290  H1
```

```
pKvlwkk..EEgnIpvsrA..........RiLHdEKslEiSNItpTdegTyvceaHnNvg  331  D1
pDvlwrrTASGgnmpLRKFSWLHSASGRVHVl.EdrslkLDDvtLEdmgeytceaDnAvg       D2
pQITwkr..KNEPmpvTra..........YiAKdNrGlRiERvQpSdegeyvcYaRnPAg  303  CE
pTvRwrk..DDgELpKsrY..........Ei.RddHTlkiRKvtAGdmgSytcVaEnMvg  337  H1
```

```
        >IG #4
QiSaRaSlIvhappNfTKrpSnKKvGlNgVvQLPcMaSgnpPpSvfwTkegVSTlMfpn.  388  D1
GiTaTGIltvhappKfvIrpKnqLvEIgDEvLfecQaNgHpRpTLYwsVegNSSllLpGy       D2
TLeasaHlRvqappSfQTkpAdqSvPAggtAtfecTLVgQpSpaYfwskegQqDllfpsy  363  CE
KAeasaTltvqEppHfvVkpRdqVvalgrtvtfQceaTgnpqpaIfwRRegsqnllf.sy  396  H1
```

```
                        qIvaQgrtvtfPceTKgnpqpavfwQkegsqnllfpn.            H2


...SsHGrQYvAADgtlQitDvrqedegyyv.cSaFSvvDssTVrVFlQvSS..vD....      440  D1
RDGRMEVTLTPEGRSVlSiARFAredSgKVvTcNalnAvgsVSsrTVVSvDt..QF....           D2
VSADGRTK..vsptgtltiEEvrqVdegAyv.cAGMnSagsslskaAlKvttKAvTGNTP     420  CE
qpPQsSsrFsvsQtgdltitnvqrsdVgyyi.cqTlnvagsiITkaYlevtd..vIA...     450  H1
qpQQPNsrCsvsptgdltitnIqrsdAgyyi.cqalTvagsilAkaQlevtd..vLT...          H2


>IG #5
erpppiiQIgpAnqtlpKgsVaTlpcratgNpSpRiKwFHdgHAvQA.GNRYSi.iqG..     496  D1
eLpppiieqgpvnqtlpvKsIVvlpcrTLgTpvpQVswYLdgIpidVqEHERrNLsDA..          D2
AKpppTieHgHQnqtlMvgsSaIlpcQaSgKpTpGiswlRdgLpidITd..sri.sqHST     477  CE
drpppViRqgpvnqtVavdgtFvlScVatgSpvpTiLwRkdgVLvSTqd..sriK.qLeN     507  H1
drpppiiLqgpAnqtlavdgtaLcKcKatgDpLpViswlkEgFTFPGRd..PrATiq.eQ          H2


                            >FN #1
SslRVDdlq.lsdSgtytciasGeRgeTswAaTltveKpgs..TSLHraAdpstypAppg     553  D1
gAlTiSdlqrHEdEgLytcvasnRNgKsswsGylRLDTptNpNiKfFrapElstypgppg          D2
gslHiAdl.kKPdtgVytciaKneDgestwsaSltveDHtsN.AqfVrMpdpsNFpsSpT     535  CE
gvlqiR.YAklGdtgRytciasTPsgeatwsayIEvQeFgVp.VqPPrPTdpNLIpsAps     565  H1
gTlqiKNl.rIsdtgtytcvaTSSsgeaswsaVlDvTeSgAT.i...SKNYdlsDLpgpps         H2


TpKvLnvsrtsISlRwAKSqEKPGAVgpIi.gyTVeyfspdlQTgwIVAaHrvGDtQVti     612  D1
kpqMvEKGEnsvtlsw...TRSNKVggSSLVgyVieMfGKNETDgwVAvGTrvQNttFtQ          D2
QpIIvnvtDtEvElHw...NAPSTsgaGpitgyiiQyYspdlgQTwFNIPDYvAStEyRi     592  CE
kpEvtdvsrnTvtlsw...qpNLNsgaTp.tSyiieafsHASgSswqtvaENvktEtSAi     621  H1
kpqvtdvtKnsvtlsw...qpGTPGTLpA.SAyiieafsQSVSNswqtvaNHvkttLytV          H2


                            >FN #2
SglTpgtsyVflvraenTQgisvpsGLsNViktIEA....DfDAASANdlsAarT.llTg     667  D1
TglLpgVNyFfliraenSHgLsLpsPMsEpitVGTR....YfNS..gLdlsEarASllsg          D2
kglkpSHsyMfViraenEkgiGTpsVSsALvttSKPAAQVAlSDKNKMdMAIaEKRlTsE     652  CE
kglkpnAiylflvraAnAYgisDpsqIsDpvktQDV.....lPTSQgVdHKQVQRE.lGN     675  H1
RglRpntiylfMvraInPkV.svT.q                                            H2


KSvelIDasAinAsavrlEwMLHvSADEkyvegLRiHyK..DaSVPSAQYHSITvMDAsa     725  D1
DvvelSnasvVDstsMKlTwQI...INGkyvegFyVYArQLpNPLNTKyRMLTILNGGGa          D2
QLIKlEEVKTinstavrlFwKKR..KLEELiDgyyiKWrGPpRTNDNQyVN...vTSpsT     707  CE
```

6

```
AvLHlHnPTvLSsssIEVHwT..vDQQSQyiQgyKiLyrPSGaNHGESDWLVFEvRTpAK  733 H1
```

```
                              >FN #3
esFvvGnlKkytKyeffLTpf...fETiegQpsnskTaltYedvpsappDNIQiGmYn..  780 D1
SsCTiTGlVQytLyeffIVpf...YKsVegKpsnsRIaRtledvpsEApYgMEALLln..      D2
eNYvvSnlMPFtnyeffVIpYHSGVHsiHgapsnsMDVltAeAPpsLppEDvRiRmlnL.  766 CE
NsVviPDlRkGVnyeIKARpf...fNEFQgaDsEIkFaKtleEApsappQgvTVSKNDGN  790 H1
```

```
QtaGWvRwTpppSQHHngNlYgykiEVSAgnTM.....KVlAnMtLnaTtTsvLlNnltt  835 D1
SSaVFLKwkapELKDRHgVlLNyH.vivRgIDtAHNFSRIlTnVtIdaASPTLvlAnltE      D2
.tTLRIswkapKAdGIngIlKgFQiviv.gQAPNNNR.....nItTnERAAsvTlFHlVt  819 CE
GtaILvswQpppEdTQngMVQEykV.WCLgnEtR.....YHInKtVdGStFsvvIPFlVP  844 H1
```

```
               <
gAVysvrLNSFtKagDgpysKpISlFMdpTHHVHPpRAHPsGTHDGRHEGqDLTYHNNgN  895 D1
gVMyTvGvaaGNnagvgpyCVpATlRldpITKRLDpFINQRDHVND.............      D2
gMTyKIrvAARSnGgvgv.........ShgTSEVIMNqDTlEKHL.AAQqENESFLYgL  868 CE
gIRysvEvaaStGagSgvKsEpQFIQldAhgNPVSpEDqVslAQQI.............  890 H1
```

```
          >          TM          <
iPPGDINPTTHKKTTdYlSGpwLMViVCiVlLvlVisAAIsM.vyFkrkhQmTKElGHLS  954 D1
................vlTqpwFIiiLgAilavlMLs..fGAMvFVkrkhMm..MkQsAL      D2
iNK.............SHVpVIViVaILiIFvViiIAY.CYwRNS.rNSD...gkDRSF  909 CE
.............SdvVKqp..AFiagiGAaCWiiLMVfsIwLyRHrkKR..NglTsTY  932 H1
```

```
VVSDNEIT.....................AlniNSKESL.wIDHHRGwRTADTDKD..  988 D1
AGIRKVPSFTFTPTVTYQRGGEAVSSGGRPGLlniSEPAAQPwLAD..TwPNTGNNHNDC  990 H1
```

```
........SgLsEsKlLSHVNSSQ..SnynnS..........DGGtDyAEvd....TRNL 1024 D1
SISCCTAGNgNsDsNlTTYSRPADCIAnynnQLDNKQTNLMLPEStVyGDvdLSNKINEM 1050 H1
```

```
                    CYTOPLASMIC MOTIF #1
TtfYNCR.......KSPDNptpyattMIiGTS........sSETCTkT.TSISADkDSGT 1068 D1
KtfNSPNLKDGRFVNPSGQptpyattQLiQSNLSNNMNNGsGDSGEkHWKPLGQQkQEVA 1110 H1
```

```
HSPyS........DAFAGQVPAVpVV..KSNyLqYPVEP..................... 1097 D1
PVQyNIVEQNKLNKDYRANDTVPpTIPYNQSyDqNTGGSYNSSDRGSSTSGSQGHKKGAR 1170 H1
```

```
        CYTOPLASMIC MOTIF #2
.........InwSEFlppppEhppp...sSTy......GyAqGSp.............. 1124 D1
TPKVPKQGGMnwADLlppppAhpppHSNsEEyNISVDESyDqEMpCPVPPARMYLQQDEL 1230 H1


..eSSRKSSKSAGSgISTNQSILNAsIHsSSSGGFsAWGVSPQYAVAcp........... 1171 D1
EEeEDERGPTPPVRgAASSPAAVSYsHQsTATLTPsPQEELQPMLQDcpEETGHMQHQPD 1290 H1


...............pENVy...sNpl.....SAVAGGTQNRYQITPTNQHPPQl.... 1203 D1
RRRQPVSPPPPPRPISpPHTyGYIsGplVSDMDTDAPEEEEDEADMEVAKMQTRRlLLRG 1350 H1


....paY...............FATTGPGGAVPPNHLP.............faTQRHaa 1230 D1
LEQTpaSSVGDLESSVTGSMINGWGSASEEDNISSGRSSVSSSDGSFFTDADfaQAVAaa 1410 H1


SeyQaglNAar...............cAQSRACNsCdALATPSPmq............. 1261 D1
Aey.aglKVarRQMQDAAGRRHFHASQcPRPTSPVsTdSNMSAAVmqKTRPAKKLKHQPG 1469 H1


        CYTOPLASMIC MOTIF #3
..........ppppvpVpEGWYQPVHPNSH.PMHpTS.SNHQIYQCSSECsDHSRSsQS 1307 D1
HLRRETYTDDLppppvpPpAIKSPTAQSKTQLEVRpVVVPKLPSMDARTDRsSDRKGsSY 1529 H1


HKrQL................QLEeHGSSAkQrgGHHRRrA.pVVQPCMESeN......ENM  D1
KGrEVLDGRQVVDMRTNPGDPREAQeQQNDGkGrgNKAAKrDLpPAKTHLIQeDILPYCRPTF  H1


LAEYEQrQYTsDCCNssrEGDTC..........SCSeGSCl..yAeAgePAPRQMTAKNT 1395 D1
PTSNNPrDPSsSSSMssrGSGSRQREQANVGRRNIAeMQVlGGy.eRgeDNNEELEETES 1651 H1
```

Exemplary such Robo specific immunogenic and/or antigenic peptides are shown in Table 2.

Table 2. Immunogenic Robo polypeptides eliciting Robo-specific rabbit polyclonal antibody:
Robo polyeptide-KLH conjugates immunized per protocol described below.

Robo Polypetide, Sequence	Immunogenicity
SEQ ID NO:2, residues 68-77	+++
SEQ ID NO:2, residues 79-94	+++
SEQ ID NO:2, residues 95-103	+++
SEQ ID NO:2, residues 122-129	+++
SEQ ID NO:2, residues 165-176	+++

SEQ ID NO:2, residues 181-191   +++

SEQ ID NO:2, residues 193-204   +++

SEQ ID NO:2, residues 244-251   +++

SEQ ID NO:2, residues 274-290   +++

SEQ ID NO:2, residues 322-331   +++

SEQ ID NO:2, residues 339-347   +++

SEQ ID NO:2, residues 407-417   +++

SEQ ID NO:2, residues 441-451   +++

SEQ ID NO:2, residues 453-474   +++

SEQ ID NO:2, residues 502-516   +++

SEQ ID NO:2, residues 541-553   +++

SEQ ID NO:2, residues 617-629   +++

In addition, species-specific antigenic and/or immunogenic peptides are readily apparent as
diverged extracellular or cytosolic regions in Table 1. Exemplary such human specific
peptides are shown in Table 3.

Table 3. Immunogenic Robo polypeptides eliciting human Robo-specific rabbit polyclonal
antibody: Robo polyeptide-KLH conjugates immunized per protocol described below (some
antibodies show cross-reactivity with corresponding mouse/rat Robo polypeptides).

Robo Polypetide, Sequence   Immunogenicity

SEQ ID NO:8, residues 1-12   +++

SEQ ID NO:8, residues 18-28   +++

SEQ ID NO:8, residues 31-40   +++

SEQ ID NO:8, residues 45-65   +++

SEQ ID NO:8, residues 106-116   +++

SEQ ID NO:8, residues 137-145   +++

SEQ ID NO:8, residues 174-184   +++

SEQ ID NO:8, residues 214-230   +++

SEQ ID NO:8, residues 274-286   +++

SEQ ID NO:8, residues 314-324   +++

SEQ ID NO:8, residues 399-412   +++

SEQ ID NO:8, residues 496-507   +++

SEQ ID NO:8, residues 548-565   +++

SEQ ID NO:8, residues 599-611   +++

SEQ ID NO:8, residues 660-671   +++

SEQ ID NO:8, residues 717-730   +++

SEQ ID NO:8, residues 780-791   +++

SEQ ID NO:8, residues 835-847   +++

SEQ ID NO:8, residues 877-891   +++

SEQ ID NO:8, residues 930-942   +++

SEQ ID NO:8, residues 981-998   +++

SEQ ID NO:8, residues 1040-1051   +++

SEQ ID NO:8, residues 1080-1090   +++

SEQ ID NO:8, residues 1154-1168   +++

SEQ ID NO:8, residues 1215-1231   +++

SEQ ID NO:8, residues 1278-1302   +++

SEQ ID NO:8, residues 1378-1400   +++

SEQ ID NO:8, residues 1460-1469   +++

SEQ ID NO:8, residues 1497-1519   +++

SEQ ID NO:8, residues 1606-1626   +++

SEQ ID NO:8, residues 1639-1651   +++

SEQ ID NO:10, residues 5-16   +++

SEQ ID NO:10, residues 38-47   +++

SEQ ID NO:10, residues 83-94   +++

SEQ ID NO:10, residues 112-125   +++

SEQ ID NO:10, residues 168-180   +++

SEQ ID NO:10, residues 195-209   +++

SEQ ID NO:10, residues 222-235   +++

SEQ ID NO:10, residues 241-254   +++

In a particular embodiment, expressed sequence tags EST;yu23d11, Accession #H77734 and EST;yq76e12, Accession #H52936, as well as peptides conceptually encoded thereby, are not within the scope of the present invention (Tables 4 and 5). In a particular

embodiment, the subject Robo polypeptides exclude the corresponding regions of the

disclosed natural human Robo I polypeptide, i.e. SEQ ID NO:8, residues 168-217 and SEQ ID

NO:8, residues 1316-1485.

Table 4 EST:yu23d11 sequences compared to H-Robo1. yu23d11 refers to the fragment of

DNA which was sequenced. The fragment was sequenced from both ends generating the

following two sequences: H77734 and H77733. yu23d11 is an unspliced cDNA. Only bases

59-215 match the coding sequence of H-Robo1 (502-651). The remaining bases are intronic.

No bases of H77733 match the coding sequence of H-Robo1.

```
LRDDFRQNPSDVMVAVGEPAVMECQPPRGHPEPTISWKKDGSPLDDKDER    H-Robo1
LRDDFRQKPSDVMVAVGEPAVMECQPPRGHPEPTISWKKDGSPLDDKDER    EST H77734
```

There is an error in the sequence, a T to G change which results in the amino acid N being

replaced by K. The sequence is shown below and has been reversed for clarity:

```
TACTTCGGGATGACTTCAGACAAAAACCTTCGGATGTCATGGTTGCAGTA    H-Robo1
TACTTCGGGATGACTTCAGACAAAACCCTTCGGATGTCATGGTTGCAGTA    EST H77734
   L   R   D   D   F   R   Q   K   P   S   D   V   M   V   A   V
                                   N
```

Table 5 EST:yq76e12 sequences compared to H-Robo1. yq76e12 refers to the fragment of

DNA which was sequenced. The fragment was sequenced from both ends generating the

following two sequences: H52936 and H52937 (the latter has been reversed for clarity). The

sequences can be seen to overlap in the middle. A gap indicates a frameshift error. Note that

errors only occur in one sequence at any one position.

```
GPLVSDMDTDAPEEEEDEADMEVAKMQTRRLLLRGLEQTPASSV    H-Robo1
GPLVSDMDTDAPEEEEDEADMEVAKMQT.RLLLRGLEQTPASSV    EST H52936
```

```
GDLESSVTGSMINGWGSASEEDNISSGRSSVSSSDGSFFTDADF    H-Robo1
GDLESSVTGSMINGWGSASEEDNISSGRSSVSSSDGSFFTDADF    EST H52936
```

```
AQAVAAA  AEYAGLKVARRQMQDA  AGR  RHFH  AS  QC  PRPT    H-Robo1
AQAVAAA  AEYAGLKVARRQMQDA  AGR  RHFH  AF  QC  PRPT    EST  H52936
     ?AAT  A?YAGLKVARRQMRDA  AGR  RHFH  AS  QC  PRPT    EST  H52937


SPVSTDSNMSAAVMQKTRPAKKLKHQPGHLRRETYTDDLPPPPV          H-Robo1
SPVFTDSNM                                            EST  H52936
SPVSTDSNMSAAVMQKTRPAKKLKHQPGHLRRETYTDDLPPPPV          EST  H52937


PPPAIKSPTAQSKTQLEVRPVVVPKLPSMDARTDK                  H-Robo1
PPPAIKSPTAQSKTQLEVRPVVVPKLPSMDARTDK                  EST  H52937
```

The subject domains provide Robo domain specific activity or function, such as Robo-specific cell, especially neuron modulating or modulating inhibitory activity, Robo-ligand-binding or binding inhibitory activity. Robo-specific activity or function may be determined by convenient *in vitro*, cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. Binding assays encompass any assay where the molecular interaction of a Robo polypeptide with a binding target is evaluated. The binding target may be a natural intracellular binding target, a Robo regulating protein or other regulator that directly modulates Robo activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a Robo specific agent such as those identified in screening assays such as described below. Robo-binding specificity may be assayed by binding equilibrium constants (usually at least about $10^7 M^{-1}$, preferably at least about $10^8 M^{-1}$, more preferably at least about $10^9 M^{-1}$), by the ability of the subject polypeptide to function as negative mutants in Robo-expressing cells, to elicit Robo specific antibody in a heterologous host (e.g a rodent or rabbit), etc.

The claimed Robo polypeptides are isolated or pure: an "isolated" polypeptide is unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample and a pure polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. A polypeptide, as used herein, is a polymer of amino acids, generally at least 6 residues, preferably at least about 10 residues, more preferably at least about 25 residues, most

preferably at least about 50 residues in length. The Robo polypeptides and polypeptide

domains may be synthesized, produced by recombinant technology, or purified from

mammalian, preferably human cells. A wide variety of molecular and biochemical methods

are available for biochemical synthesis, molecular expression and purification of the subject

compositions, see e.g. Molecular Cloning, A Laboratory Manual (Sambrook, *et al.* Cold

Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, *et al.*,

Greene Publ. Assoc., Wiley-Interscience, NY) or that are otherwise known in the art.

The invention provides binding agents specific to the claimed Robo polypeptides,

including natural intracellular binding targets, etc., methods of identifying and making such

agents, and their use in diagnosis, therapy and pharmaceutical development. For example,

specific binding agents are useful in a variety of diagnostic and therapeutic applications,

especially where pathology, wound repair incompetency or prognosis is associated with

improper or undesirable axon outgrowth, orientation or inhibition thereof. Novel Robo-

specific binding agents include Robo-specific receptors, such as somatically recombined

polypeptide receptors like specific antibodies or T-cell antigen receptors (see, e.g Harlow and

Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory), natural

intracellular binding agents identified with assays such as one-, two- and three-hybrid screens,

non-natural intracellular binding agents identified in screens of chemical libraries such as

described below, etc. Agents of particular interest modulate Robo function.

In a particular embodiment, the subject polypeptides are used to generate Robo- or

human Robo-specific antibodies. For example, the Robo- and human Robo-specific peptides

described above are covalently coupled to keyhole limpet antigen (KLH) and the conjugate is

emulsified in Freunds complete adjuvant. Laboratory rabbits are immunized according to

conventional protocol and bled. The presence of Robo-specific antibodies is assayed by solid

phase immunosorbant assays using immobilized Robo polypeptides of SEQ ID NO:2, 4, 6, 8,

10 or 12. Human Robo-specific antibodies are characterized as uncross-reactive with non-

human Robo polypeptides (SEQ ID NOS:2, 4, 6 and 12).

Accordingly, the invention provides methods for modulating cell function comprising

the step of modulating Robo activity, e.g. by contacting the cell with a Robo inhibitor, e.g.

inhibitory Robo deletion mutants, Robo-specific antibodies, etc. (supra). The target cell may

reside in culture or in situ, i.e. within the natural host. The inhibitor may be provided in any

convenient way, including by (i) intracellular expression from a recombinant nucleic acid or

(ii) exogenous contacting of the cell. For many in situ applications, the compositions are added to a retained physiological fluid such as blood or synovial fluid. For CNS administration, a variety of techniques are available for promoting transfer of the therapeutic across the blood brain barrier including disruption by surgery or injection, drugs which transiently open adhesion contact between CNS vasculature endothelial cells, and compounds which facilitate translocation through such cells. Robo polypeptide inhibitors may also be amenable to direct injection or infusion, topical, intratracheal/nasal administration e.g. through aerosol, intraocularly, or within/on implants e.g. fibers e.g. collagen, osmotic pumps, grafts comprising appropriately transformed cells, etc. A particular method of administration involves coating, embedding or derivatizing fibers, such as collagen fibers, protein polymers, etc. with therapeutic proteins. Other useful approaches are described in Otto et al. (1989) J Neuroscience Research 22, 83-91 and Otto and Unsicker (1990) J Neuroscience 10, 1912-1921. Generally, the amount administered will be empirically determined, typically in the range of about 10 to 1000 µg/kg of the recipient and the concentration will generally be in the range of about 50 to 500 µg/ml in the dose administered. Other additives may be included, such as stabilizers, bactericides, etc. will be present in conventional amounts. For diagnostic uses, the inhibitors or other Robo binding agents are frequently labeled, such as with fluorescent, radioactive, chemiluminescent, or other easily detectable molecules, either conjugated directly to the binding agent or conjugated to a probe specific for the binding agent.

The amino acid sequences of the disclosed Robo polypeptides are used to back-translate Robo polypeptide-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural Robo-encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc, Madison WI). Robo-encoding nucleic acids used in Robo-expression vectors and incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional studies such as the efficacy of candidate drugs for disease associated with Robo-modulated cell function, etc.

The invention also provides nucleic acid hybridization probes (Tables 6, 7) and replication / amplification primers (Tables 7, 8) having a Robo cDNA specific sequence comprising SEQ ID NO:1, 3, 5, 7, 9 or 11 and sufficient to effect specific hybridization

thereto (i.e. specifically hybridize with SEQ ID NO:1, 3, 5, 7, 9 or 11, respectively, in the presence of CDO cDNA.

Table 5. Hybridisation Probes for Human Roundabout 1

Immunoglobulin Domain #1

CCACCTCGCATTGTTGAACACCCTTCAGACCTGATTGTCTCAAAAGGAGAACCTGCAACTTTGAACTGCAAAGCT
GAAGGCCGCCCCACACCCACTATTGAATGGTACAAAGGGGGAGAGAGAGTGGAGACAGACAAAGATGACCCTCGC
TCACACCGAATGTTGCTGCCGAGTGGATCTTTATTTTTCTTACGTATAGTACATGGACGGAAAAGTAGACCTGAT
GAAGGAGTCTATGTCTGTGTAGCAAGGAATTACCTTGGAGAGGCTGTGAGCCACAATGCATCGCTGGAAGTAGCC
ATA

Immunoglobulin Domain#2

CTTCGGGATGACTTCAGACAAAACCCTTCGGATGTCATGGTTGCAGTAGGAGAGCCTGCAGTAATGGAATGCCAA
CCTCCACGAGGCCATCCTGAGCCCACCATTTCATGGAAGAAAGATGGCTCTCCACTGGATGATAAAGATGAAAGA
ATAACTATACGAGGAGGAAAGCTCATGATCACTTACACCCGTAAAAGTGACGCTGGCAAATATGTTTGTGTTGGT
ACCAATATGGTTGGGGAACGTGAGAGTGAAGTAGCCGAGCTGACTGTCTT

Immunoglobulin Domain #3

AGAGAGACCATCATTTGTGAAGAGACCCAGTAACTTGGCAGTAACTGTGGATGACAGTGCAGAATTTAAATGTGA
GGCCCGAGGTGACCCTGTACCTACAGTACGATGGAGGAAAGATGATGGAGAGCTGCCCAAATCCAGATATGAAAT
CCGAGATGATCATACCTTGAAAATTAGGAAGGTGACAGCTGGTGACATGGGTTCATACACTTGTGTTGCAGAAAA
TATGGTGGGCAAAGCTGAAGCATCTGCTACTCTGACTGTTCAAGAACC

Immunoglobulin Domain #4

CCACATTTTGTTGTGAAACCCCGTGACCAGGTTGTTGCTTTGGGACGGACTGTAACTTTTCAGTGTGAAGCAACC
GGAAATCCTCAACCAGCTATTTTCTGGAGGAGAGAAGGGAGTCAGAATCTACTTTTCTCATATCAACCACCACAG
TCATCCAGCCGATTTTCAGTCTCCCAGACTGGCGACCTCACAATTACTAATGTCCAGCGATCTGATGTTGGTTAT
TACATCTGCCAGACTTTAAATGTTGCTGGAAGCATCATCACAAAGGCATATTTGGAAGTTACAGATGTGATTGCA

Immunoglobulin Domain #5

GATCGGCCTCCCCCAGTTATTCGACAAGGTCCTGTGAATCAGACTGTAGCCGTGGATGGCACTTTCGTCCTCAGC
TGTGTGGCCACAGGCAGTCCAGTGCCCACCATTCTGTGGAGAAAGGATGGAGTCCTCGTTTCAACCCAAGACTCT
CGAATCAAACAGTTGGAGAATGGAGTACTGCAGATCCGATATGCTAAGCTGGGTGATACTGGTCGGTACACCTGC
ATTGCATCAACCCCCAGTGGTGAAGCAACATGGAGTGCTTACATTGAAGTTCAAGAATTTG

Fibronectin Domain #1

GAGTTCCAGTTCAGCCTCCAAGACCTACTGACCCAAATTTAATCCCTAGTGCCCCATCAAAACCTGAAGTGACAG

ATGTCAGCAGAAATACAGTCACATTATCGTGGCAACCAAATTTGAATTCAGGAGCAACTCCAACATCTTATATTA

TAGAAGCCTTCAGCCATGCATCTGGTAGCAGCTGGCAGACCGTAGCAGAGAATGTGAAAACAGAAACATCTGCCA

TTAAAGGACTCAAACCTAATGCAATTTACCTTTTCCTTGTGAGGGCAGCTAATGCATATGGAATTAGTGATC

Fibronectin Domain #2

CAAGCCAAATATCAGATCCAGTGAAAACACAAGATGTCCTACCAACAAGTCAGGGGGTGGACCACAAGCAGGTCC

AGAGAGAGCTGGGAAATGCTGTTCTGCACCTCCACAACCCCACCGTCCTTTCTTCCTCTTCCATCGAAGTGCACT

GGACAGTAGATCAACAGTCTCAGTATATACAAGGATATAAAATTCTCTATCGGCCATCTGGAGCCAACCACGGAG

AATCAGACTGGTTAGTTTTTGAAGTGAGGACGCCAGCCAAAAACAGTGTGGTAATCCCTGATCTCAGAAAGGGAG

TCAACTATGAAATTAAGGCTCGCCCTTTTTTTAATGAATTTCAAGGAGCAG

Fibronectin Domain #3

ATAGTGAAATCAAGTTTGCCAAAACCCTGGAAGAAGCACCCAGTGCCCCACCCCAAGGTGTAACTGTATCCAAGA

ATGATGGAAACGGAACTGCAATTCTAGTTAGTTGGCAGCCACCTCCAGAAGACACTCAAAATGGAATGGTCCAAG

AGTATAAGGTTTGGTGTCTGGGCAATGAAACTCGATACCACATCAACAAAACAGTGGATGGTTCCACCTTTTCCG

TGGTCATTCCCTTTCTTGTTCCTGGAATCCGATACAGTGTGGAAGTGGCAGCCAGCACTGGGGCTGGGTCTGGGG

TAAAG

Transmembrane Domain

AGATTTCAGATGTGGTGAAGCAGCCGGCCTTCATAGCAGGTATTGGAGCAGCCTGTTGGATCATCCTCATGGTCT

TCAGCATCTGGCTTTATCGACACCG

Cytoplasmic Motif #1

AATCTGAAGGATGGGCGTTTTGTCAATCCATCAGGGCAGCCTACTCCTTACGCCACCACTCAGCTCATCCAGTCA

AACCTCAGCAACAACATGAACAATG

Cytoplasmic Motif #2

CCCAAGGTACCAAAACAGGGTGGCATGAACTGGGCAGACCTGCTTCCTCCTCCCCCAGCACATCCTCCTCCACAC

AGCAATAGCGAAGAGTACAACATTT

Cytoplasmic Motif #3

CCAGCCAGGACATCTGCGCAGAGAAACCTACACAGATGATCTTCCACCACCTCCTGTGCCGCCACCTGCTATAAA

GTCACCTACTGCCCAATCCAAGACA

Table 6. Hybridisation Probes for Human Roundabout 2

Immunoglobulin Domain #4

CAGATTGTTGCTCAAGGTCGAACAGTGACATTTCCCTGTGAAACTAAAGGAAACCCACAGCCAGCTGTTTTTTGG

CAGAAAGAAGGCAGCCAGAACCTACTTTTCCCAAACCAACCCCAGCAGCCCAACAGTAGATGCTCAGTGTCACCA

ACTGGAGACCTCACAATCACCAACATTCAACGTTCCGACGCGGGTTACTACATCTGCCAGGCTTTAACTGTGGCA

GGAAGCATTTTAGCAAAAGCTCAACTGGAGGTTACTGATGTTTTGACA

Immunoglobulin Domain #5

GATAGACCTCCACCTATAATTCTACAAGGCCCAGCCAACCAAACGCTGGCAGTGGATGGTACAGCGTTACTGAAA

TGTAAAGCCACTGGTGATCCTCTTCCTGTAATTAGCTGGTTAAAGGAGGGATTTACTTTTCCGGGTAGAGATCCA

AGAGCAACAATTCAAGAGCAAGGCACACTGCAGATTAAGAATTTACGGATTTCTGATACTGGCACTTATACTTGT

GTGGCTACAAGTTCAAGTGGAGAGGCTTCCTGGAGTGCAGTGCTGGATGTGACAGAGTCT

Fibronectin Domain #1

GGAGCAACAATCAGTAAAAACTATGATTTAAGTGACCTGCCAGGGCCACCATCCAAACCGCAAGTCACTGATGTT

ACTAAGAACAGTGTCACCTTGTCCTGGCAGCCAGGTACCCCTGGAACCCTTCCAGCAAGTGCATATATCATTGAG

GCTTTCAGCCAATCAGTGAGCAACAGCTGGCAGACCGTGGCAAACCATGTAAAGACCACCCTCTATACTGTAAGA

GGACTGCGGCCCAATACAATCTACTTATTCATGGTCAGAGCGATCAACCCCAAGGTYTCAGTGACCCAAGT

Table 7. Primer Pairs for PCR of Human Roundabout 1 Domains

Immunoglobulin Domain #1

```
Forward: 5' CCACCTCGCATTGTTGAACACCCTTCAGAC 3'
Reverse: 5' ATGGCTACTTCCAGCGATGCATTGTGGCTC 3'
```

Immunoglobulin Domain #2

```
Forward: 5' CTTCGGGATGACTTCAGACAAAACCCTTCG 3'
Reverse: 5' TAAGACAGTCAGCTCGGCTACTTCACTCTC 3'
```

Immunoglobulin Domain #3

```
Forward: 5' AGAGAGACCATCATTTGTGAAGAGACCCAG 3'
Reverse: 5' AGGTTCTTGAACAGTCAGAGTAGCAGATGC 3'
```

Immunoglobulin Domain #4

```
Forward: 5' CCACATTTTGTTGTGAAACCCCGTGACCAG 3'
Reverse: 5' TGCAATCACATCTGTAACTTCCAAATATGC 3'
```

17

Immunoglobulin Domain #5

```
Forward: 5' ATCGGCCTCCCCCAGTTATTCGACAAGGTC 3'
Reverse: 5' CAAATTCTTGAACTTCAATGTAAGCACTCC 3'
```

Fibronectin Domain #1

```
Forward: 5' GAGTTCCAGTTCAGCCTCCAAGACCTACTG 3'
Reverse: 5' TCACTAATTCCATATGCATTAGCTGCCCTC 3'
```

Fibronectin Domain #2

```
Forward: 5' CAAGCCAAATATCAGATCCAGTGAAAACAC 3'
Reverse: 5' ATCTGCTCCTTGAAATTCATTAAAAAAAGG 3'
```

Fibronectin Domain #3

```
Forward: 5' ATAGTGAAATCAAGTTTGCCAAAACCCTG 3'
Reverse: 5' CTCTTTACCCCAGACCCAGCCCCAGTGCTG 3'
```

Transmembrane Domain

```
Forward: 5' GGACCAAGTCAGCCTCGCTCAGCAGATTTC 3'
Reverse: 5' ACTAGTAAGTCCGTTTCTCTTCTTGCGGTG 3'
```

Cytoplasmic Motif #1

```
Forward: 5' CTGAAGGATGGGCGTTTTGTCAATCCATC 3'
Reverse: 5' GTCCCAGTGGTTTCCAGTGCTTCTCGCCAG 3'
```

Cytoplasmic Motif #2

```
Forward: 5' GGCACAAGAAAGGGGCAAGAACACCCAAGG 3'
Reverse: 5' ATAGCTTTCATCTACAGAAATGTTGTACTC 3'
```

Cytoplasmic Motif #3

```
Forward: 5' ACCAGACCAGCCAAGAAACTGAAACACCAG 3'
Reverse: 5' GTACTTCCAGCTGTGTCTTGGATTGGGCAG 3'
```

Table 8. Human Roundabout 2 Primer Pairs

Immunoglobulin Domain #4

```
Forward:  5'  GTTGCTCAAGGTCGAACAGTGACATTTCCC  3'
Reverse:  5'  TGTCAAAACATCAGTAACCTCCAGTTGAGC  3'
```


Immunoglobulin Domain #5

```
Forward:  5'  GATAGACCTCCACCTATAATTCTACAAGGC  3'
Reverse:  5'  GACTCTGTCACATCCAGCACTGCACTCCAG  3'
```


Fibronectin Domain #1

```
Forward:  5'  CAATCAGTAAAAACTATGATTTAAGTG  3'
Reverse:  5'  TCGCTCTGACCATGAATAAGTAGATTG  3'
```


Such primers or probes are at least 12, preferably at least 24, more preferably at least 36 and most preferably at least 96 bases in length. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M NaPO$_4$, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C. Robo nucleic acids can also be distinguished using alignment algorithms, such as BLASTX (Altschul *et al.* (1990) Basic Local Alignment Search Tool, J Mol Biol 215, 403-410).

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e. unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. The subject recombinant nucleic acids comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9 or 11, or fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by (i.e. contiguous with) a sequence other than that which it is joined to on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, more preferably fewer than 500 bp, which is at a terminus or is immediately flanked by a sequence other than that which it is joined to on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is

19

often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

In a particular embodiment, expressed sequence tags EST;yu23d11, Accession #H77734 and EST;yq76e12, Accession #H52936, and deletion mutants thereof, are not within the scope of the present invention. In another embodiment, the subject Robo nucleic acids exclude the corresponding regions of the disclosed natural human Robo I nucleic acids, i.e. SEQ ID NO:7, nucleotides 500-651 and SEQ ID NO:7, nucleotides 3945-4455.

Table 10. Exemplary differences between H52936 and corresponding human Robo I sequences.

(1) At position 86, there is a T instead of an A. The new codon therefore reads TGA (Stop) instead of AGA (R).

(2) There is a missing G at position 286-7, causing a frameshift.

(3) There is an extra G at position 334, causing a frameshift.

(4) There is an extra T at position 344, causing a frameshift.

(5) There is an extra N at position 357, causing a frameshift.

(6) There is a T instead of a C at 362. The new codon reads TTT (F) instead of TCT (S).

(7) There is an extra T at position 364, causing a frameshift.

(8) There is an extra N at position 370, causing a frameshift and a changed amino acid (the codon TTN is ambiguous).

(9) There are two Ts at position 394 and 395 instead of a C, causing a frameshift and amino acid changes.

Table 11 . Exemplary differences between H52937 (reverse sequence) and corresponding human Robo I sequences.

(1) There are multiple errors in the first 30 bases.

(2) At position 63, a G replaces an A. The new codon CGG codes for R instead of CAG for Q.

(3) The EST ends by joining to part of the human glycophorin B gene (353-442)

The subject nucleic acids find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of Robo genes and gene transcripts and in detecting or amplifying

nucleic acids encoding additional Robo homologs and structural analogs. In diagnosis, Robo hybridization probes find use in identifying wild-type and mutant Robo alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic Robo nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active Robo.

The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of a Robo modulatable cellular function. Generally, these screening methods involve assaying for compounds which modulate Robo interaction with a natural Robo binding target. A wide variety of assays for binding agents are provided including labeled *in vitro* protein-protein binding assays, immunoassays, cell based assays, etc. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

Cell and animal based neural guidance/repulsion assays are described in detail in the experimental section below. *In vitro* binding assays employ a mixture of components including a Robo polypeptide, which may be part of a fusion product with another peptide or polypeptide, e.g. a tag for detection or anchoring, etc. The assay mixtures comprise a natural intracellular Robo binding target. While native full-length binding targets may be used, it is frequently preferred to use portions (e.g. peptides) thereof so long as the portion provides binding affinity and avidity to the subject Robo polypeptide conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds; preferably small organic compounds and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents may also be included in the mixture. These include reagents like salts, buffers, neutral proteins, e.g. albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc. may be used.

The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the Robo polypeptide specifically binds the cellular

binding target, portion or analog with a reference binding affinity. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening.

After incubation, the agent-biased binding between the Robo polypeptide and one or more binding targets is detected by any convenient way. Where at least one of the Robo or binding target polypeptide comprises a label, the label may provide for direct detection as radioactivity, luminescence, optical or electron density, etc. or indirect detection such as an epitope tag, etc. A variety of methods may be used to detect the label depending on the nature of the label and other assay components, e.g. through optical or electron density, radiative emissions, nonradiative energy transfers, etc. or indirectly detected with antibody conjugates, etc.

A difference in the binding affinity of the Robo polypeptide to the target in the absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates the binding of the Robo polypeptide to the Robo binding target. For example, in the cell-based assay also described below, a difference in Robo-dependent modulation of axon outgrowth or orientation in the presence and absence of an agent indicates the agent modulates Robo function. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least a 90% difference.

The following experimental section and examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Cloning of the *roundabout* Gene. The *robo¹* allele was mapped to the *plexus-brown* interval on the right arm of the second chromosome by recombination mapping; the numbers of recombinants suggested a map position very close to *plexus* at 58F/59A. One deficiency [*Df(2R)P*, which deletes 58E3/F1 through 60D14/E2] fails to complement *robo* mutations, two other deficiencies [*Df(2R)59AB* and *Df(2R)59AD*, which delete 59A1/3 through 59B1/2 and 59A1/3 through 59D1/4 respectively] do complement *robo*, and a duplication [*Dp(2;Y)bw⁺Y*, which duplicates 58F1/59A2 through 60E3/F1] rescues *robo* mutations. This mapping places *robo* in the 58F/59A region.

22

We initiated chromosomal walks from P1 clones mapped to the region, beginning from the distal side using clone DS02204 and from the proximal side using clone DS05609. We used cosmid clones (Tamkun et al., 1992) to complete a walk of ~150 kb. We then looked for RFLPs in the recombinants between the multiple marked chromosome and the *robo* mutant chromosome. A 6.8kb EcoRI fragment from cosmid 106-5 identified a HindII RFLP on the mapping chromosome that was present on a single *robo* mutant recombinant line. This fragment identified a proximal limit for the location of *robo*. Further deficiencies in this region were then tested (Kerrebrock et al., 1995). Of these deficiencies, *Df(2R)X58-5* and *Df(2R)X58-12* remove *robo* while *Df(2R)X58-1* does not. *Df(2R)X58-12* fails to complement *Df(2R)59AB* yet complements *Df(2R)59AD* indicating that *Df(2R)59AB* extends further proximal; this proximal endpoint provides a distal limit for the location of *robo*. Probes from the walk were used to identify the breakpoints of these deficiencies (Figure 1A). *Df(2R)X58-1* breaks in a 9.6 kb EcoRI/BamHI fragment within cosmid GJ12, whereas *Df(2R) 59AB* breaks in a 8 kb BamHI/EcoRI fragment within cosmid 106-1435. This reduces the location of *robo* to a 75 kb region bounded by these restriction fragments. Hybridization of 0-16 hr poly-A$^+$ embryonic Northern blots with cosmids GJ12, 106-12, and 106-1435 revealed at least five transcripts. Reverse Northern mapping identified the regions containing these transcripts (Figure 1A). These regions were used as probes to isolate cDNAs. Seven different cDNAs were isolated and analyzed by in situ hybridization. The expression pattern of five of these transcripts allowed us to tentatively discount them as encoding for *robo* since they were not expressed in the embryonic CNS at the appropriate stage. Of the two cDNAs remaining, 12-1 appeared by its size and expression the most likely candidate for *robo*. A 16 kb XbaI fragment including the 12-1 transcript and a region 5' to the transcript is capable of rescuing the *robo* mutant.

roundabout Encodes a Member of the Immunoglobulin Superfamily. We recovered and sequenced overlapping cDNA clones corresponding to the 12-1 transcription unit. A single long open reading frame (ORF) that encodes 1395 amino acids was identified (D1 in Table 1). Conceptual translation of the ORF reveals the Robo protein to be a member of the Ig superfamily; Robo's ectodomain contains five immunoglobulin (Ig)-like repeats followed by three fibronectin (Fn) type-III repeats. The predicted ORF also contains a transmembrane domain and a large 457 amino acid (a.a.) cytoplasmic domain. Hydropathy analysis of the Robo sequence indicates a single membrane spanning domain of 25 a.a. (Kyte and Doolittle,

1982) plus a signal sequence with a predicted cleavage site between G51 and Q52 (Nielsen et al 1997).

We identify the 12-1 transcript as encoding *robo* based on several criteria. First, the embryonic *robo* phenotype can be rescued by the 16 kb XbaI genomic fragment containing this cDNA; no other transcripts are contained in this 16 kb XbaI fragment. Second, we identified a CfoI RFLP associated with the allele *robo^6*. This polymorphism is due to a change of nucleotide 332 of the ORF from G to A, which results in a change of Gly$_{111}$ to Asp. Gly111 is in the first Ig domain (Figure 2), and is conserved in all Robo homologues identified. The change is specific to the allele *robo^6* and is not seen in the parental chromosome or in any of the other seven alleles, all of which were generated from the same parental genotype. Third, the production of antibodies (below) which recognize the Robo protein reveals that the alleles *robo^1*, *robo^2*, *robo^3*, *robo^4* and *robo^5* do not produce Robo protein (Table 12).

Table 12. *robo* Mutant Alleles

Allele	Synonym	Class
robo^1	GA285	Protein null
robo^2	GA1112	Protein null
robo^3	Z14	Protein null
robo^4	Z570	Protein null
robo^5	Z1772	Protein null
robo^6	Z1757	Protein positive; Gly$_{111}$ to Asp
robo^7	Z2130	Reduced protein levels
robo^8	Z3127	Protein positive

All alleles were generated by EMS mutagenesis of *FasIII* null chromosomes. Each of these alleles appear to represent a complete, or near complete, loss-of-function phenotype for *robo*, since the mutant phenotype observed when these alleles are placed over a chromosome deficient for the *robo* locus [Df(2R) X58-5] is indistinguishable from the homozygous allele.

Finally, transgenic neural expression of *robo* rescues the midline crossing phenotype of *robo* mutants (see below).

Developmental Northern blot analysis using both cDNA and genomic probes suggests that *robo* is encoded by a single transcript of ~7500 bp. We sequenced genomic DNA and identified 17 introns within the sequence of which 14 are only 50-75 bp in length plus three

introns of 843 bp, 236 bp, and 110 bp (Figure 1B). The precise start point of the transcript has not been determined.

A Family of Evolutionarily Conserved Robo-like Proteins. The presence of five Ig and three Fn domains, a transmembrane domain, and a long (452 a.a.) cytoplasmic region indicates that Robo may be a receptor and signaling molecule. The netrin receptor DCC/Frazzled/UNC-40 has a related domain structure, with 6 Ig and 4 Fn domains and a similarly long cytoplasmic region (Keino-Masu et al., 1996; Chan et al., 1996; Kolodziej et al., 1996). The only currently known protein with a "5 + 3" organization is CDO (Kang et al., 1997). However, CDO is only distantly related to Robo (15-33% a.a. identity between corresponding Ig and FN domains).

We identified other "5 + 3" proteins in vertebrates whose amino acid identity exceeds that of CDO and represent Robo homologues. A human expressed sequence tag (EST; yu23d11, Accession #H77734) shows high homology to the second Ig domain of *robo* and was used to probe a human fetal brain cDNA library (Stratagene). The clones recovered correspond to a human gene with five Ig and three Fn domains (Figure 2). Exemplary functional Robo domains are listed in Tables 13-17 (the corresponding encoding nucleic acids are readily discernable from the corresponding nucleic acid sequences of Sequence Listing).

Table 13. Exemplary domains of human Robo 1, by amino acid sequence positions

Signal sequence:	6-21
First Immunoglobulin domain:	68-167
Second Immunoglobulin domain:	168-258
Third Immunoglobulin domain:	259-350
Fourth Immunoglobulin domain:	351-450
Fifth Immunoglobulin domain:	451-546
First Fibronectin domain:	547-644
Second Fibronectin domain:	645-761
Third Fibronectin domain:	762-862
Transmembrane domain:	896-917
Cytoplasmic motif #1:	1070-1079
Cytoplasmic motif #2:	1181-1195
Cytoplasmic motif #3:	1481-1488

Table 14. Exemplary domains of human Robo II, by amino acid sequence positions

Fourth Immunoglobulin domain: 1-91

Fifth Immunoglobulin domain: 92-185

First Fibronectin domain: 186-282

Table 15. Exemplary domains of drosophila Robo 1, by amino acid sequence positions

Signal sequence: 30-46

First Immunoglobulin domain: 56-152

Second Immunoglobulin domain: 153-251

Third Immunoglobulin domain: 252-344

Fourth Immunoglobulin domain: 345-440

Fifth Immunoglobulin domain: 441-535

First Fibronectin domain: 536-635

Second Fibronectin domain: 636-753

Third Fibronectin domain: 754-854

Transmembrane domain: 915-938

Cytoplasmic motif #1:   1037-1046

Cytoplasmic motif #2:   1098-1119

Cytoplasmic motif #3:   1262-1269

Table 16. Exemplary domains of drosophila Robo II, by amino acid sequence positions

Immunoglobulin domain #1:   4-99

Immunoglobulin domain #2:   100-192

Immunoglobulin domain #3:   193-296

Immunoglobulin domain #4:   297-396

Immunoglobulin domain #5:   397-494

Fibronectin domain #1:   495-595

Fibronectin domain #2:   596-770

Fibronectin domain #3:   771-877

Transmembrane domain: 906-929

Conserved cytoplasmic motif #1:   1075-1084

26

Table 17. Exemplary domains of C. elegans Robo 1, by amino acid sequence positions

First Immunoglobulin domain:	30-129
Second Immunoglobulin domain:	130-223
Third Immunoglobulin domain:	224-315
Fourth Immunoglobulin domain:	316-453
Fifth Immunoglobulin domain:	454-543
First Fibronectin domain:	544-643
Second Fibronectin domain:	644-766
Third Fibronectin domain:	767-865
Transmembrane domain:	900-922
Cytoplasmic motif #1:	1036-1045
Cytoplasmic motif #2:	1153-1163
Cytoplasmic motif #3:	1065-1074

The homology is particularly high in the first two Ig domains (58% and 48% a.a. identity respectively, compared to 26% and 30% for the same two Ig domains between D-Robo1 and CDO) and together with the overall identity throughout the extracellular region and the presence of three conserved cytoplasmic motifs has led us to designate this as the human *roundabout 1* gene (*H-robo1*). Database searching reveals a nucleotide sequence corresponding to *H-robo1* in the database, *DUTT1*, which differs in the signal sequence suggesting alternative splicing, a 9 bp insertion and seven single base pair changes. Five ESTs (see Experimental Procedures) show high sequence similarity to the cytoplasmic domain of *H-robo1*. Sequencing of cDNAs isolated using one of these ESTs as a probe confirmed a second human *roundabout* gene (*H-robo2*).

Degenerate PCR primers based on conserved sequences between *H-robo1* and *D-robo1* were used to isolate a PCR fragment from a rat embryonic E13 brain cDNA library. The fragment was used to probe an E13 spinal cord cDNA library, resulting in the isolation of a full length Rat *robo* gene (*R-robo1*). The predicted protein shows high sequence identitiy (>95%) with *H-robo1* over the entire length. The 5' sequences of different *R-robo1* cDNA clones indicates that this gene is alternatively spliced in a similar fashion to *H-robo1/DUTT1*. We used a similar approach to isolate cDNA clones for *R-robo2*, which is highly homologous to *H-robo2*.

The mouse EST vi92e02 is highly homologous to the cytoplasmic portion of *H-robo1*. The *C. elegans Sax-3* gene is also a *robo* homologue (Table 1; Zallen et al., 1997). A second Drosophila *robo* gene (*D-robo2*) is also predicted from analysis of genomic sequence in the public database. Taken together these data indicate that Robo is the founding member of a new subfamily of Ig superfamily proteins with at least one member in nematode, two in Drosophila, two in rat, and two in human.

The alignment of the Robo family proteins reveals that the first and second Ig domains are the most highly conserved portion of the extracellular domain. The cytoplasmic domains are highly divergent except for the presence of three highly conserved motifs (Table 18).

Table 18. Conserved Cytoplasmic Motifs: Amino acid alignments of the three conserved cytoplasmic motifs are shown below the structure; in C.elegans *robo*, motifs #2 and #3 have been switched to provide a better alignment.

Conserved Cytoplasmic Motif #1

```
PDNPTPYATTMIIGTSS    1050  Drosophila roundabout-I

SGQPTPYATTQLIQSNL    1083  Human roundabout-I

NASPAPYATSSILSPHQ    1088  Drosophila roundabout-II

HDDPSPYATTTLVLSNQ    1049  C.elegans roundabout

   PtPYATT.hh....           Consensus (where h is I, L or V)
```

Conserved Cytoplasmic Motif #2

```
INWSE.FLPPPPEHPPPSSTYG.Y    1119  Drosophila roundabout-I

MNWAD.LLPPPPAHPPPHSNSEEY    1202  Human roundabout-I

STWANVPLPPPPVQPLPGTELEHY      31  Human roundabout-II

KTLMD.FIPPPPSNPPPP.GGHVY    1168  C.elegans roundabout-I

nW...hhPPPP.PPP.s....Y Consensus (where h is hydrophobic)
```

Conserved Cytoplasmic Motif #3

```
PSPMQPPPPVPVPEGW.Y    1273  Drosophila roundabout-I

YTDDLPPPPVPPPAIKSP    1493  Human roundabout-I

YADDLPPPPVPPPAIKSP      90  Mouse roundabout-I
```

```
RAPAMPTNPVPPEPPARY  1077  C.elegans  roundabout

......PPPPVPPP....             Consensus
```

The consensus for the first motif is PtPYATTxhh, where x is any amino acid and h is I, L, or V. The presence of a tyrosine in the center of the motif indicates a site for phosphorylation. The other two motifs consist of runs of prolines separated by one or two amino acids and are reminiscent of binding sites for SH3 domains. In particular, the LPPP sequence in motif #2 provides a good binding site for the Drosophila Enabled protein or its mammalian homologue Mena (Niebuhr et al., 1997). All three of these conserved sites can function as binding sites for domains (e.g. SH3 domains) of linker/adapter proteins functioning in Robo-mediated signal transduction.

Robo is Regionally Expressed on Longitudinal Axons in the Drosophila Embryo. In order to determine the role that *robo* might play in regulating axon crossing behavior, we examined the *robo* expression pattern in the embryonic CNS. The in situ hybridization pattern of *robo* mRNA in Drosophila shows it to have elevated and widespread expression in the CNS. We raised a monoclonal antibody (MAb 13C9) against part of the extracellular portion (amino acids 404-725) of the protein to visualize Robo expression. Robo is first seen in the embryo weakly expressed in lateral stripes during germband extension. At the onset of germband retraction, Robo expression is observed in the neuroectoderm. By the end of stage 12, as the growth cones first extend, Robo is seen on growth cones which project ipsilaterally, including pCC, aCC, MP1, dMP2, and vMP2. Strikingly, little or no Robo expression is observed on commissural growth cones as they extend towards and across the midline. However, as these growth cones turn to project longitudinally, their level of Robo expression dramatically increases. Robo is expressed at high levels on all longitudinally-projecting growth cones and axons. In contrast, Robo is expressed at nearly undetectable levels on commissural axons. This is striking since ~90% of axons in the longitudinal tracts also have axon segments crossing in one of the commissures. Thus, Robo expression is regionally restricted. Robo expression is also seen at a low level throughout the epidermis and at a higher level at muscle attachment sites. In stage 16-17 embryos, faint Robo staining can be seen in the commissures but at levels much lower than observed in the longitudinal tracts.

Immunoelectron Microscopy of Robo. We used immunoelectron microscopy to examine Robo localization at higher resolution. In stage 13 embryos, Robo is expressed at

higher levels on growth cones and filopodia in the longitudinal tracts than on the longitudinal axons themselves. This localization is consistent with the model that Robo functions as a guidance receptor. The increased sensitivity of immunoelectron microscopy reveals the presence of very low levels of Robo protein on the surface of commissural axons. In addition, Robo-positive vesicles can be seen inside the commissural axons, possibly representing transport of Robo to the growth cone. Finally, by reconstructing the path of single axons by use of serial sections, we confirm that Robo expression is greatly up-regulated after individual axons turn from the commissure into a longitudinal tract. The expression of Robo on non-crossing and post-crossing axons and its higher level of expression on growth cones and its filopodia, provide a model where Robo functions as an axon guidance receptor for a repulsive midline cue.

Transgenic Expression of Robo. We hypothesized that if Robo is indeed a growth cone receptor for a midline repellent, then pan-neural expression of Robo protein during the early stages of axon outgrowth might lead to a *robo* gain-of-function phenotype similar to the *comm* loss-of-function and opposite of the *robo* loss-of-function. To test this hypothesis, we cloned a *robo* cDNA containing the complete ORF but lacking most of its untranslated regions (UTRs) downstream of the UAS promoter in the pUAST vector and generated transgenic flies for use in the GAL4 system (Brand and Perrimon, 1993). Expression of *robo* in all neurons was achieved by crossing the *UAS-robo* flies to either the *elav-GAL4* or *scabrous-GAL4* lines.

Surprisingly, pan-neural expression of *robo* mRNA did not produce a strong axon scaffold phenotype as assayed with MAb BP102. Staining with anti-Fas II (MAb 1D4) revealed subtle fasciculation defects, but overall the axon scaffold looked quite normal. An insight into why we failed to observe a stronger *robo* ectopic expression phenotype was provided by staining these embryos with the anti-Robo MAb. Interestingly, the Robo protein, although expressed at higher levels than in wild type, remains restricted as in wild type, i.e., high levels of expression on the longitudinal portions of axons and very low levels on the commissures. This result indicates that there must be strong regulation of Robo expression, probably post-translational, that assures its localization to longitudinal axon segments. Such a mechanism could operate by the regulation of protein translation, transport, insertion, internalization and/or stability.

We used these transgenic flies to rescue *robo* mutants. Expression of *robo* by the *elav-*

GAL4 line in both *robo³* and *robo⁵* homozygotes rescued the midline crossing of Fas II

positive axons including pCC and other identified neurons.

Robo Appears to Function in a Cell Autonomous Fashion. To test whether Robo can

function in a cell autonomous fashion, we used the *UAS-robo* transgene with the *ftz$_{ng}$-GAL4*

line (Lin et al., 1994) . The *ftz$_{ng}$-GAL4* line expresses in a subset of CNS neurons, including

many of the earliest neurons to be affected by the *robo* mutation such as pCC, vMP2, dMP2,

and MP1. Expression of *robo* by the *ftz$_{ng}$-GAL4* line is sufficient to rescue these identified

neurons in the *robo* mutant: pCC, which in robo mutants heads towards and crosses the

midline, in these rescued embryos now projects ipsilaterally and does not cross the midline.

When the same embryos were stained with the anti-robo MAb 13C9, we observed that all

Robo-positive axons did not cross the midline. The *ftz$_{ng}$-GAL4* line drives expression in many

of the axons in the pCC pathway (Lin et al., 1994), a medial longitudinal fascicle. In *robo*

mutants, this axon fascicle freely crosses and circles the midline, joining with its contralateral

pathway. When rescued by the *ftz$_{ng}$-GAL4* line driving UAS-*robo*, this pathway now largely

remains on its own side of the midline, even though occasionally a few axons cross the

midline. These experiments support the notion that Robo can function in a cell autonomous

fashion.

Expression of Mammalian *robo1* in the Rat Spinal Cord. The isolation of several

vertebrate Robo homologues suggests that Robo may play a similar role in orchestrating

midline crossing in the vertebrate nervous system as it does in Drosophila. In the vertebrate

spinal cord, the ventral midline is comprised of a unique group of cells called the floor plate

(for review, Colamarino and Tessier-Lavigne, 1995). As in the Drosophila nervous system,

the vertebrate spinal cord contains both crossing and non-crossing axons. Spinal commissural

neurons are born in the dorsal half of the spinal cord; commissural axons project to and cross

the floor plate before turning longitudinally in a rostral direction. In contrast, the axons of two

other classes of neurons, dorsal association neurons and ventral motor neurons, do not cross

the floor plate (Altman and Bayer, 1984).

To address the possibility that Robo may play a role in organizing the projections of

these spinal neurons, we examined the expression of rat *robo1* by RNA in situ hybridization.

A rat *robo1* riboprobe spanning the first three Ig domains was hybridized to transverse

sections of E13 rat spinal cord. At E13, when many commissural axons will have already

extended across the floor plate (Altman and Bayer, 1984), rat *robo1* is expressed at high levels

in the dorsal spinal cord, in a pattern corresponding to the cell bodies of commissural neurons. Rat *robo1* is also expressed at lower levels in a subpopulation of ventral cells in the region of the developing motor column. Interestingly, this expression pattern is similar to and overlaps partly with the mRNA encoding DCC, another Ig superfamily member which is also expressed on commissural and motor neurons and encodes a receptor for Netrin-1 (Keino-Masu et al, 1996). Rat *robo1* is not, however, expressed in the either the floor plate or the roof plate of the spinal cord or in the dorsal root ganglia. This is in contrast to rat *cdo*, which is strongly expressed in the roof plate (KB, MT-L, and R. Krauss. In the periphery, rat *robo1* is also found to be expressed in the the myotome and developing limb, in a pattern reminiscent of *c-met* (Ebens et al, 1996), indicating that rat *robo1* may also be expressed by migrating muscle precursor cells. Therefore, like its Drosophila homologue, rat *robo1* RNA is expressed by both crossing and non-crossing populations of axons, indicating that it encodes the functional equivalent of D-Robo1.

Genetic Stocks. All eight independent *robo* alleles were isolated on chromosomes deficient for *Fasciclin III* as described in Seeger et al., 1993. Subsequent use of a duplication that includes *FasIII*, and recombination of the *robo* chromosomes, indicates that the *robo* phenotype is independent of the absence of *FasIII*. Deficiencies were obtained from the Drosophila stock center at Bloomington, Indiana.

Cloning and Molecular Analysis of the *robo* Genes. Start points for a molecular walk to *robo* were obtained from the Berkeley and Crete Drosophila Genome Projects. Chromosomal walking was performed using standard techniques to isolate cosmids from the Tamkun library (Tamkun et al., 1992). cDNAs were isolated from the Zinn 9-12 hour Drosophila embryo gt11 library (Zinn et al., 1988), and from a human fetal brain library (Stratagene). Northern blot of poly-A$^+$ RNA and reverse Northern blots were hybridized using sensitive Church conditions.

Sequencing of the cDNAs and genomic subclones was performed by the dideoxynucleotide chain termination method using Sequenase (USB) following the manufacturer's protocol and with the AutoRead kit or AutoCycle kit (Pharmacia) or by ^{33}P cycle sequencing. Reactions were analyzed on a Pharmacia LKB or ABI automated laser fluorescent DNA sequencers respectively. The cDNAs were sequenced completely on both strands. Sequence contigs were compiled using Lasergene, Intelligenetics, and AssemblyLIGN software (Kodak Eastman). Database searches were performed using BLAST

(Altschuel et al., 1990).

A full length *D-robo1* cDNA was generated by ligating two partial cDNAs at an internal HpaI site and subcloning into the EcoRI site of pBluescript.SK+. A full length *H-robo1* cDNA was synthesized by ligating an XbaI-SalI fragment from a cDNA and a PCR product coding for the carboxy-terminal 222 amino acids at a SalI site. The PCR product has an EcoRI site introduced at the stop codon. The ligation product was cloned into pBluescript.SK+ digested with XbaI and EcoRI.

To clone the rat *robo1* cDNA, degenerate oligonucleotide primers designed against sequences conserved between the 5' ends of D-Robo1 and H-Robo1 were used to amplify a 500 bp fragment from an E13 rat brain cDNA by PCR. This fragment was used to screen an E13 spinal cord library at high stringency, resulting in the isolation of a 4.2 kb cDNA clone comprising all but the last 700 nucleotides. Subsequent screenings of the library with non-overlapping probes from this cDNA led to the isolation of 4 partial and 7 full length clones. To clone the rat *robo2* cDNA, we screened the same library with a fragment of the *H-robo2* cDNA.

Expressed Sequence Tag and Genomic Sequences. The ESTs yu23d11 (#H77734), zr54g12 (#AA236414) and yq76e12 (#H52936, #H52937) code for portions of H-Robo1. The EST yq7e12 is aberrantly spliced to part of the human glycophorinB gene. Five ESTs yn50a07, yg02b06, yg17b06, yn13a04 and ym17g11 code for part of *H-robo2*. The Drosophila P1 clone DS00329 encodes the genomic sequence of *D-robo2*. Sequences 1825710 and 1825711 (both: #U88183; locus ZK377) code for the predicted sequence of C. elegans *robo*. The EST vi62e02 (#AA499193) codes for mouse *robo1*.

Identification of Molecular Defects In *robo* Alleles. Southern blots of *robo* alleles and their parental chromosomes were hybridized with fragments from the genomic cosmid clone 106-1435 or partial cDNA clones to identify restriction fragment length polymorphisms affecting the *robo* transcription unit. DNA was obtained from homozygous mutant embryos. 35 cycles of the PCR was subsequently performed on the DNA obtained from half an embryo. Primers specific for the region flanking the CfoI polymorphism used were : ROBO6 (5'-GCATTGGGTCATCTGTAGAG -3') and ROBO23 (5'-AGCTATCTGGAGGGAGGCAT-3'). The PCR products were purified on a Pharmacia H300 spin column and sequenced directly.

Transformation of Drosophila, *robo* Rescue, and Overexpression. The 16 kb XbaI

33

fragment from cosmid 106-1435 was cloned into the Drosophila transformation vector pCaSpeR3. Transformant lines were generated and mapped by standard procedures. Four independent lines were shown to rescue *robo*[1,3,5] alleles as judged by MAb 1D4 staining.

PCR amplification of the D-robo ORF using the primers (5'-GAGTGGTGAATTCAACAGCACCAAAACCACAAAATGCATCCC-3') and (5'-CGGGGAGTCTAGAACACTTCATCCTTAGGTG-3') produced a PCR product with an altered ribosome binding site that more closely matches the Drosophila consensus (Cavener, 1987), and has only 21bp of 5' UTR and no 3' UTR sequences. The PCR product was digested with EcoRI and XbaI and cloned into pBluescript (Stratagene) and subsequently, pUAST (Brand and Perrimon 1993). Transformant lines were crossed to *elav-GAL4* and *sca-GAL4* lines which express GAL4 in all neurons, or *ftzng-GAL4* which expresses in a subset of CNS neurons (Lin et al, 1994). Embryos were assayed by staining with MAbs BP102, 1D4 and 13C9. For ectopic expression in the *robo* mutant background, the stocks *robo*[3] and *robo*[5] (both protein nulls) were used. Crosses utilized the stocks *w; robo/CyO; UAS-robo* and *w; robo/CyO; elav-GAL4*. Due to the difficulty of maintaining a balanced stock, *robo/+; ftz-ngGAL4/+* males were generated as required.

Generation of Fusion Proteins and Antibodies. A six histidine tagged fusion protein was constructed by cloning amino acids 404-725 of the D-robo protein into the PstI site of the pQE31 vector (Qiagen). Fusion proteins were purified under denaturing conditions and subsequently dialyzed against PBS. Immunization of mice and MAb production followed standard protocols (Patel, 1994).

RNA Localization and Protein Immunocytochemistry. Digoxigenin labeled antisense *robo* transcripts were generated from a subclone of a *robo* cDNA in Bluescript. In-situ tissue hybridization was performed as described in Tear et al., 1996. Immunocytochemistry was performed as described by Patel, 1994. MAb 1D4 was used at a dilution of 1:5 and BP102 at 1:10. For anti-robo staining, MAb 13C9 was diluted 1:10 in PBS with 0.1% Tween-20, and the embryos were fixed and cracked so as to minimize exposure to methanol. The presence of triton and storage of embryos in methanol were both found to destroy the activity of MAb 13C9.

In situ hybridization of rat spinal cords was carried out essentially as described in Fan and Tessier-Lavigne, 1994. E13 embryos were fixed in 4% paraformaldehyde, processed, embedded in OCT, and sectioned to 10 m. A 1.0kb ^{35}S antisense rRobo riboprobe spannning

the the first three immunoglobulin domains was used for hybridization. An additional non-overlapping probe was also used with identical results. DCC transcripts were detected as described in Keino-Masu et al., 1996. Immunohistochemistry against TAG-1 was carried out on 10 m transverse spinal cord sections using 4D7 monoclonal antibody (Dodd et al, 1988).

Electron Microscopy. Canton S embryos were hand devitellinized, opened dorsally to remove the gut, and prepared for immunoelectron microscopy according to the procedures described previously (Lin et al., 1994), with the following modifications. The fixed embryos were incubated sequentially with MAb 13C9 (1:1) for 1-2 hours, biotinylated goat anti-mouse secondary antibody (1:250) for 1.5 hours, and then streptavidin-conjugated HRP (1:200) for 1.5 hours. Hydrogen peroxide (0.01%) was used instead of glucose oxidase for the HRP-DAB reaction.

References

Altman, J. and Bayer, S.A. (1984) Adv. Anat. Embryol. Cell Biol. 85, 1-164.

Altschul, S.F., et al. (1990) J. Mol. Biol. 215, 403-410.

Bastiani, M.J., et al. (1987) Cell 48, 745-755.

Brand, A. H., and Perrimon, N. (1993) Development 118, 401-415.

Cavener, D. (1987) Nucl. Acids Res. 15, 1353-1361.

Chan, S. et al. (1996) Cell 87, 187-195.

Dodd, J., et al. (1988) Neuron 1, 105-116.

Ebens, A., et al. (1996) Neuron 17, 1157-1172.

Elkins, T., et al. (1990) Cell 60, 565-575.

Fan, C.M. and Tessier-Lavigne, M. (1994) Cell 79, 1175-1186.

Gertler, F.B., et al. (1995) Genes Develop. 9, 521-533.

Harris, R., Sabatelli, L.M., and Seeger, M.A. (1996) Neuron 17, 217-228.

Hedgecock, E.M., Culotti, J.G., and Hall, D.H. (1990) Neuron 4, 61-85.

Kang, J-S., et al. (1997) J. Cell Biol. 138, 203-213.

Keino-Masu, K., et al. (1996) Cell 87, 175-185.

Kennedy, T.E., et al. (1994) Cell 78, 425-435.

Kerrebrock, A. W., et al. (1995) Cell 83, 247-256.

Kidd, T., Russell, C., Goodman, C.S., and Tear, G. (1997). Dosage sensitive and complementary functions of Roundabout and Commissureless control axon crossing of the CNS midline. Neuron, in review.

Kolodziej, P.A., et al. (1996) Cell 87, 197-204.

Kyte, J., and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105-132.

Lin, D.M., et al. (1994) Neuron 13, 1055-1069.

Mitchell, K.J., et al. (1996) Neuron 17, 203-215.

Myers, P.Z., and Bastiani, M.J. (1993) Journal of Neuroscience 13, 127-143.

Niebuhr, K., et al. (1997) EMBO J. 16, 5433-5444.

Nielsen, H., et al. (1997) Protein Engineering 10, 1-6.

Patel, N. H. (1994) In "Methods in Cell Biology, Vol 44. Drosophila melanogaster: Practical

Uses in Cell Biology" (L. S. B. Goldstein and E. Fyrberg, eds) Academic Press, New York.

Seeger, M., Tear, G., Ferres-Marco, D., and Goodman C.S. (1993) Neuron 10, 409-426.

Serafini, T., et al. (1994) Cell 78, 409-424.

Stoeckli, E.T., and Landmesser, L.T. (1995) Neuron 14, 1165-1179.

Stoeckli, et al. (1997) Neuron 18, 209-221.

Tamkun, J.W., et al. (1992) Cell 68, 561-572.

Tear, G., et al. (1993) Perspectives on Developmental Neurobiology 1, 183-194.

Tear G., et al. (1996) Neuron 16, 501-514.

Tessier-Lavigne, M., and Goodman, C.S. (1996) Science 274, 1123-1133.

Wadsworth, W.G., Bhatt, H., and Hedgecock, E.M. (1996) Neuron 16, 35-46.

Zallen, J., Yi, A., and Bargmann, C. (1997). The conserved immunoglobulin superfamily

member SAX-3/Robo directs multiple aspects of axon guidance in C. elegans. Cell, in

review.

Zinn, K., McAllister, L., and Goodman, C. S. (1988)Cell 53, 577-587.

All publications and patent applications cited in this specification are herein
incorporated by reference as if each individual publication or patent application were
specifically and individually indicated to be incorporated by reference. Although the
foregoing invention has been described in some detail by way of illustration and example for
purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the
art in light of the teachings of this invention that certain changes and modifications may be
made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1.   An isolated Robo polypeptide comprising SEQ ID NO:2, 4, 6, 8, 10 or 12, or a polypeptide domain thereof having at least 12 consecutive residues thereof and a Robo-specific activity, wherein said domain is encoded by neither EST yq76e12 nor yq76e12.

2.   An isolated polypeptide according to claim 1, wherein said activity is selected from at least one of a Robo-competitive binding, Robo-specific antigenicity and a Robo-specific immunogenicity.

3.   An isolated polypeptide according to claim 1, wherein said domain comprises at least one of a Robo immunoglobulin, fibronectin or cytoplasmic motif domain.

4.   A recombinant nucleic acid encoding a polypeptide according to claim 1.

5.   A cell comprising a nucleic acid according to claim 4.

6.   A method of making a Robo polypeptide, comprising the following steps: incubating a host cell or cellular extract containing a nucleic acid according to claim 4 under conditions whereby the polypeptide encoded by the nucleic acid is expressed and recovering the expressed polypeptide.

7.   An isolated Robo polypeptide made by the method of claim 6.

8.   An isolated *robo* nucleic acid comprising a strand of SEQ ID NO:1, 3, 5, 7, 9 or 11, or a fragment thereof having at least 24 consecutive bases thereof, and sufficient to specifically hybridize with a nucleic acid having the sequence defined by the corresponding opposite strand, wherein the fragment is contained in neither EST yq76e12 nor yq76e12.

9. A method for modulating cell function or morphology comprising providing the cell with an agent which modulates activity of a Robo polypeptide or function of a *robo* gene, wherein the agent comprises a polypeptide according to claim 1 or a Robo-specific antibody.

SUBSTITUTE SHEET (RULE 26)
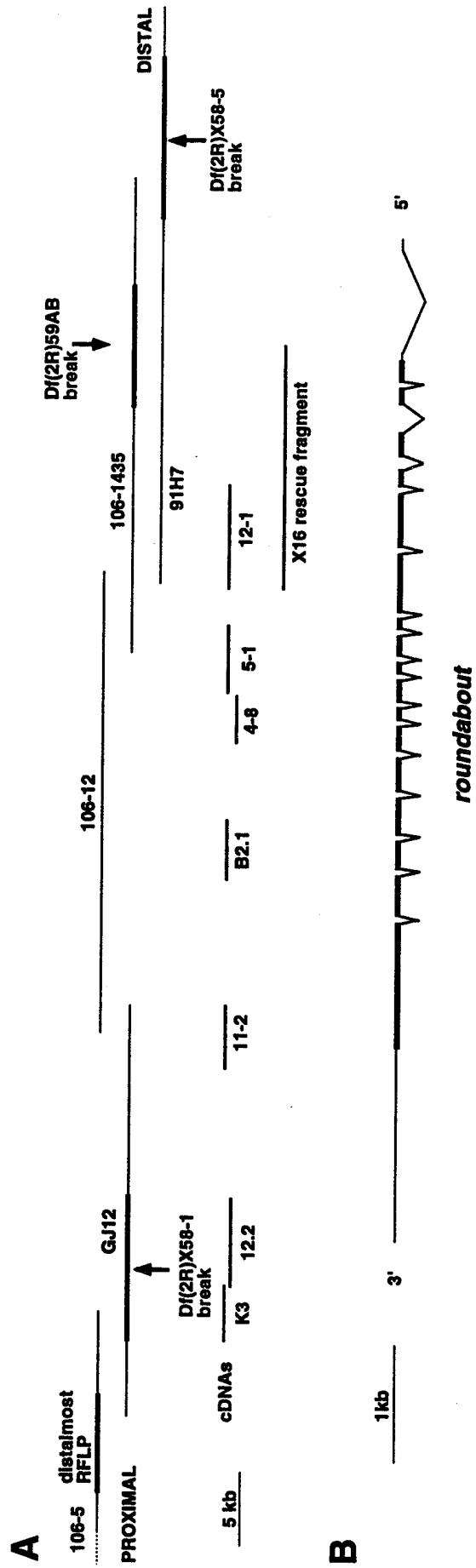
FIG. 1
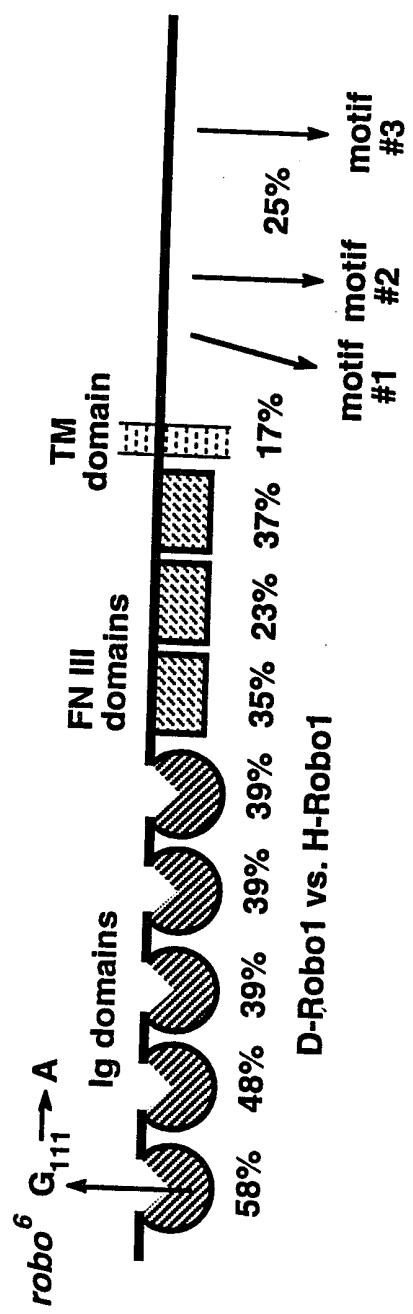
FIG. 2

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Goodman, Corey S.

                Kidd, Thomas

                Mitchell, Kevin

                Tear, Guy

    (ii) TITLE OF INVENTION: Robo: A Novel Family of Polypeptide and
           Nucleic Acids

   (iii) NUMBER OF SEQUENCES: 12

    (iv) CORRESPONDENCE ADDRESS:

        (A) ADDRESSEE: SCIENCE & TECHNOLOGY LAW GROUP

        (B) STREET: 75 DENISE DRIVE

        (C) CITY: HILLSBOROUGH

        (D) STATE: CALIFORNIA

        (E) COUNTRY: USA

        (F) ZIP: 94010

    (v) COMPUTER READABLE FORM:

        (A) MEDIUM TYPE: Floppy disk

        (B) COMPUTER: IBM PC compatible

        (C) OPERATING SYSTEM: PC-DOS/MS-DOS

        (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

    (vi) CURRENT APPLICATION DATA:

        (A) APPLICATION NUMBER:

        (B) FILING DATE:

        (C) CLASSIFICATION:

   (viii) ATTORNEY/AGENT INFORMATION:

        (A) NAME: OSMAN, RICHARD A

        (B) REGISTRATION NUMBER: 36,627

        (C) REFERENCE/DOCKET NUMBER: B98-006

    (ix) TELECOMMUNICATION INFORMATION:

        (A) TELEPHONE: (650) 343-4341

        (B) TELEFAX: (650) 343-4342

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 4188 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: double

1

SUBSTITUTE SHEET (RULE 26)

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

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

ATGCATCCCA TGCATCCCGA AAACCACGCC ATCGCCCGGA GCACGAGCAC CACTAATAAC   60

CCATCTCGCA GTCGGAGCAG CAGGATGTGG CTCCTGCCCG CCTGGCTGCT CCTCGTCCTG   120

GTGGCCAGCA ATGGCCTGCC AGCAGTCAGA GGCCAGTACC AATCGCCACG TATCATCGAG   180

CATCCCACGG ATCTGGTCGT TAAGAAGAAT GAACCCGCCA CGCTCAACTG CAAAGTGGAG   240

GGCAAGCCGG AACCCACCAT TGAGTGGTTT AAGGATGGCG AACCCGTCAG CACCAACGAA   300

AAGAAATCGC ACCGCGTCCA GTTCAAGGAC GGCGCCCTCT TCTTTTACAG GACAATGCAA   360

GGCAAGAAGG AGCAGGACGG CGGAGAGTAC TGGTGCGTGG CCAAGAACCG AGTGGGCCAG   420

GCCGTTAGTC GCCATGCCTC CCTCCAGATA GCTGTTTTGC GCGACGATTT CGCGTGGAG   480

CCCAAAGACA CGCGAGTGGC CAAAGGCGAG ACGGCTCTGC TGGAGTGTGG GCCGCCCAAA   540

GGCATTCCAG AGCCAACGCT GATTTGGATA AAGGACGGCG TTCCCTTGGA CGACCTGAAA   600

GCCATGTCGT TTGGCGCCAG CTCCCGCGTT CGAATTGTGG ACGGTGGCAA CCTGCTGATC   660

AGCAATGTGG AGCCCATTGA TGAGGGCAAC TACAAGTGCA TTGCCCAGAA TCTGGTAGGC   720

ACCCGCGAGA GCAGCTATGC CAAGCTGATT GTCCAGGTCA AACCATACTT TATGAAGGAG   780

CCCAAGGATC AGGTGATGCT CTACGGCCAG ACAGCCACTT TCCACTGCTC AGTGGGCGGT   840

GATCCGCCGC CGAAAGTGTT GTGGAAAAAG GAGGAGGGCA ATATTCCGGT GTCCAGAGCG   900

CGAATCCTTC ACGACGAGAA AGTTTAGAG ATATCCAACA TAACGCCCAC CGATGAGGGC   960

ACCTATGTCT GCGAGGCACA CAACAATGTC GGTCAGATCA GCGCTAGGGC TTCTCTTATA   1020

GTCCACGCTC CGCCGAACTT TACGAAAAGA CCCAGTAACA AGAAAGTGGG ACTAAATGGG   1080

GTTGTCCAAC TACCTTGCAT GGCCTCCGGA AACCCTCCGC CGTCTGTATT CTGGACCAAG   1140

GAAGGAGTAT CCACTCTTAT GTTCCCAAAT AGTTCGCACG GAAGGCAGTA TGTGGCTGCC   1200

GATGGAACTC TGCAGATTAC GGATGTGCGG CAGGAAGACG AAGGCTACTA TGTGTGTTCC   1260

GCTTTCAGTG TAGTCGATTC CTCTACAGTA CGGGTTTTCC TGCAAGTCAG CTCGGTAGAC   1320

GAGCGTCCAC CTCCGATTAT TCAAATCGGA CCTGCCAATC AAACACTGCC CAAGGGATCA   1380

GTTGCTACTT TACCCTGTCG GGCCACTGGA AATCCCAGTC CCGTATCAA GTGGTTCCAC   1440

GATGGACATG CCGTACAAGC GGGCAATCGA TACAGCATCA TCCAAGGAAG CTCACTGAGA   1500

GTCGATGACC TTCAACTAAG TGACTCTGGT ACCTACACCT GCACTGCATC TGGCGAACGA   1560

GGAGAAACTT CCTGGGCTGC CACACTAACG GTGGAAAAAC CCGGTTCTAC ATCTCTTCAC   1620

CGGGCAGCTG ATCCTAGCAC TTATCCTGCT CCTCCAGGAA CACCTAAAGT CCTGAATGTC   1680

AGTCGCACCA GCATTAGTCT TCGTTGGGCT AAAAGCCAAG AGAAACCCGG AGCTGTGGGC   1740

CCAATCATTG GATACACTGT AGAGTACTTC AGTCCGGATC TGCAAACTGG TTGGATTGTG   1800

GCTGCCCATC GAGTCGGCGA CACTCAAGTC ACTATCTCGG GTCTCACTCC TGGCACTTCG   1860

TATGTGTTCC TAGTTAGAGC TGAGAATACT CAGGGTATTT CTGTGCCTTC CGGCTTATCA   1920

AATGTTATTA AAACCATTGA GGCAGATTTC GATGCAGCTT CTGCCAATGA TTTGTCAGCA   1980

GCTCGAACTT TGCTGACAGG AAAGTCGGTG GAGCTAATAG ATGCCTCGGC TATCAATGCT   2040

AGTGCCGTTA GACTTGAGTG GATGCTCCAC GTGAGCGCTG ATGAGAAATA CGTAGAGGGC   2100

2

SUBSTITUTE SHEET (RULE 26)

```
CTGCGCATAC ACTATAAGGA TGCCAGTGTA CCATCCGCAC AGTATCACTC GATCACTGTT    2160
ATGGATGCCT CTGCAGAATC GTTTGTGGTG GGAAACCTTA AGAAGTACAC CAAGTATGAG    2220
TTCTTCCTAA CACCCTTTTT TGAGACAATT GAAGGACAGC CCAGTAACTC CAAGACAGCC    2280
CTCACCTATG AAGATGTTCC CTCCGCACCA CCGGATAACA TTCAGATTGG CATGTACAAC    2340
CAAACAGCCG GTTGGGTGCG TTGGACTCCG CCACCCTCCC AGCACCACAA TGGCAATTTG    2400
TATGGCTACA AGATTGAGGT CAGCGCCGGT AACACCATGA AGGTGCTGGC CAATATGACT    2460
CTTAATGCTA CCACCACATC TGTGCTCCTA AATAACCTAA CCACCGGAGC TGTGTACAGC    2520
GTGAGGTTGA ACTCCTTTAC CAAGGCAGGA GATGGACCTT ACTCCAAACC GATATCACTA    2580
TTCATGGACC CCACCCATCA TGTGCATCCG CCACGGGCAC ATCCAAGCGG CACCCATGAT    2640
GGGCGACATG AGGGACAGGA TCTCACGTAT CATAACAATG GCAACATACC ACCTGGCGAC    2700
ATTAATCCCA CCACTCATAA AAAGACCACT GACTACCTAT CTGGACCGTG GCTAATGGTG    2760
CTGGTCTGCA TCGTTCTTCT AGTCCTGGTT ATTTCGGCGG CTATTTCGAT GGTCTACTTC    2820
AAGCGCAAGC ATCAAATGAC CAAGGAATTG GGTCACTTAA GTGTGGTCAG TGACAACGAA    2880
ATAACCGCAT TAAATATCAA TAGCAAAGAG AGCCTTTGGA TAGACCATCA TCGTGGATGG    2940
CGAACTGCCG ATACTGACAA AGACTCAGGA TTAAGCGAAT CGAAGCTACT ATCCCACGTT    3000
AACAGCAGTC AATCCAACTA CAATAACTCC GATGGAGGAA CCGATTATGC AGAAGTTGAC    3060
ACCCGTAACC TTACCACCTT CTACAATTGT CGCAAGAGCC CCGATAATCC CACGCCGTAC    3120
GCCACCACTA TGATCATTGG TACCTCTTCC AGTGAGACCT GCACCAAGAC AACATCTATA    3180
AGTGCCGATA AGGACTCGGG AACTCATTCG CCCTATTCTG ACGCATTTGC CGGTCAGGTG    3240
CCAGCGGTTC CTGTTGTCAA ATCCAACTAT CTTCAGTATC CGGTTGAACC GATCAACTGG    3300
TCAGAGTTTC TACCCCCGCC GCCAGAACAC CCACCTCCGT CTTCTACCTA TGGATACGCA    3360
CAAGGATCTC CTGAATCTTC GCGGAAGAGC TCCAAAAGCG CAGGTTCCGG CATTTCTACA    3420
AATCAAAGCA TTCTGAACGC ATCCATACAC AGCAGCTCCT CGGGCGGCTT TTCAGCTTGG    3480
GGAGTATCGC CCCAATATGC TGTCGCCTGT CCACCGGAAA ACGTTTATAG CAATCCGCTG    3540
TCGGCAGTGG CTGGCGGCAC CCAGAACCGC TATCAGATAA CGCCCACAAA CCAACATCCG    3600
CCACAGTTAC CGGCCTACTT TGCCACCACG GGTCCAGGAG GAGCTGTACC ACCCAACCAC    3660
CTGCCATTTG CCACACAGCG TCATGCAGCC AGCGAGTACC AGGCTGGACT GAATGCAGCG    3720
CGATGTGCCC AAAGCCGCGC CTGCAACAGC TGCGATGCCT TGGCCACACC CTCGCCCATG    3780
CAACCCCCAC CGCCAGTTCC CGTACCCGAG GGCTGGTACC AACCGGTGCA TCCCAATAGC    3840
CACCCGATGC ACCCGACCTC CTCCAACCAC CAGATCTACC AGTGCTCCTC CGAGTGCTCG    3900
GATCACTCGA GGAGCTCGCA GAGTCACAAG CGGCAGCTGC AGCTCGAGGA GCACGGCAGC    3960
AGTGCCAAAC AACGCGGAGG ACACCACCGT CGACGAGCCC CGGTGGTGCA GCCGTGCATG    4020
GAGAGCGAGA ACGAGAACAT GCTGGCGGAG TACGAGCAGC GCCAGTACAC CAGCGATTGC    4080
TGCAATAGCT CCCGCGAGGG CGACACCTGC TCCTGCAGCG AGGGATCCTG TCTTTACGCC    4140
GAGGCGGGCG AGCCGGCGCC TCGTCAAATG ACTGCTAAGA ACACCTAA              4188
```

(2) INFORMATION FOR SEQ ID NO:2:
    (i) SEQUENCE CHARACTERISTICS:

3

SUBSTITUTE SHEET (RULE 26)

(A) LENGTH: 1395 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Met His Pro Met His Pro Glu Asn His Ala Ile Ala Arg Ser Thr Ser
1               5                   10                  15

Thr Thr Asn Asn Pro Ser Arg Ser Arg Ser Ser Arg Met Trp Leu Leu
            20                  25                  30

Pro Ala Trp Leu Leu Leu Val Leu Val Ala Ser Asn Gly Leu Pro Ala
            35                  40                  45

Val Arg Gly Gln Tyr Gln Ser Pro Arg Ile Ile Glu His Pro Thr Asp
    50                  55                  60

Leu Val Val Lys Lys Asn Glu Pro Ala Thr Leu Asn Cys Lys Val Glu
65                  70                  75                  80

Gly Lys Pro Glu Pro Thr Ile Glu Trp Phe Lys Asp Gly Glu Pro Val
            85                  90                  95

Ser Thr Asn Glu Lys Lys Ser His Arg Val Gln Phe Lys Asp Gly Ala
            100                 105                 110

Leu Phe Phe Tyr Arg Thr Met Gln Gly Lys Lys Glu Gln Asp Gly Gly
            115                 120                 125

Glu Tyr Trp Cys Val Ala Lys Asn Arg Val Gly Gln Ala Val Ser Arg
    130                 135                 140

His Ala Ser Leu Gln Ile Ala Val Leu Arg Asp Asp Phe Arg Val Glu
145                 150                 155                 160

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

Gly Pro Pro Lys Gly Ile Pro Glu Pro Thr Leu Ile Trp Ile Lys Asp
            180                 185                 190

Gly Val Pro Leu Asp Asp Leu Lys Ala Met Ser Phe Gly Ala Ser Ser
            195                 200                 205

Arg Val Arg Ile Val Asp Gly Gly Asn Leu Leu Ile Ser Asn Val Glu
    210                 215                 220

Pro Ile Asp Glu Gly Asn Tyr Lys Cys Ile Ala Gln Asn Leu Val Gly
225                 230                 235                 240

Thr Arg Glu Ser Ser Tyr Ala Lys Leu Ile Val Gln Val Lys Pro Tyr
                245                 250                 255
```

4

Phe Met Lys Glu Pro Lys Asp Gln Val Met Leu Tyr Gly Gln Thr Ala
            260   265   270

Thr Phe His Cys Ser Val Gly Gly Asp Pro Pro Pro Lys Val Leu Trp
        275   280   285

Lys Lys Glu Glu Gly Asn Ile Pro Val Ser Arg Ala Arg Ile Leu His
    290   295   300

Asp Glu Lys Ser Leu Glu Ile Ser Asn Ile Thr Pro Thr Asp Glu Gly
305   310   315   320

Thr Tyr Val Cys Glu Ala His Asn Asn Val Gly Gln Ile Ser Ala Arg
            325   330   335

Ala Ser Leu Ile Val His Ala Pro Pro Asn Phe Thr Lys Arg Pro Ser
            340   345   350

Asn Lys Lys Val Gly Leu Asn Gly Val Val Gln Leu Pro Cys Met Ala
        355   360   365

Ser Gly Asn Pro Pro Pro Ser Val Phe Trp Thr Lys Glu Gly Val Ser
    370   375   380

Thr Leu Met Phe Pro Asn Ser Ser His Gly Arg Gln Tyr Val Ala Ala
385   390   395   400

Asp Gly Thr Leu Gln Ile Thr Asp Val Arg Gln Glu Asp Glu Gly Tyr
            405   410   415

Tyr Val Cys Ser Ala Phe Ser Val Val Asp Ser Ser Thr Val Arg Val
            420   425   430

Phe Leu Gln Val Ser Ser Val Asp Glu Arg Pro Pro Pro Ile Ile Gln
        435   440   445

Ile Gly Pro Ala Asn Gln Thr Leu Pro Lys Gly Ser Val Ala Thr Leu
    450   455   460

Pro Cys Arg Ala Thr Gly Asn Pro Ser Pro Arg Ile Lys Trp Phe His
465   470   475   480

Asp Gly His Ala Val Gln Ala Gly Asn Arg Tyr Ser Ile Ile Gln Gly
            485   490   495

Ser Ser Leu Arg Val Asp Asp Leu Gln Leu Ser Asp Ser Gly Thr Tyr
        500   505   510

Thr Cys Thr Ala Ser Gly Glu Arg Gly Glu Thr Ser Trp Ala Ala Thr
        515   520   525

Leu Thr Val Glu Lys Pro Gly Ser Thr Ser Leu His Arg Ala Ala Asp
    530   535   540

Pro Ser Thr Tyr Pro Ala Pro Pro Gly Thr Pro Lys Val Leu Asn Val
545   550   555   560

5

SUBSTITUTE SHEET (RULE 26)

Ser Arg Thr Ser Ile Ser Leu Arg Trp Ala Lys Ser Gln Glu Lys Pro
              565   570   575

Gly Ala Val Gly Pro Ile Ile Gly Tyr Thr Val Glu Tyr Phe Ser Pro
              580   585   590

Asp Leu Gln Thr Gly Trp Ile Val Ala Ala His Arg Val Gly Asp Thr
              595   600   605

Gln Val Thr Ile Ser Gly Leu Thr Pro Gly Thr Ser Tyr Val Phe Leu
          610   615   620

Val Arg Ala Glu Asn Thr Gln Gly Ile Ser Val Pro Ser Gly Leu Ser
625   630   635   640

Asn Val Ile Lys Thr Ile Glu Ala Asp Phe Asp Ala Ala Ser Ala Asn
              645   650   655

Asp Leu Ser Ala Ala Arg Thr Leu Leu Thr Gly Lys Ser Val Glu Leu
              660   665   670

Ile Asp Ala Ser Ala Ile Asn Ala Ser Ala Val Arg Leu Glu Trp Met
          675   680   685

Leu His Val Ser Ala Asp Glu Lys Tyr Val Glu Gly Leu Arg Ile His
          690   695   700

Tyr Lys Asp Ala Ser Val Pro Ser Ala Gln Tyr His Ser Ile Thr Val
705   710   715   720

Met Asp Ala Ser Ala Glu Ser Phe Val Val Gly Asn Leu Lys Lys Tyr
              725   730   735

Thr Lys Tyr Glu Phe Phe Leu Thr Pro Phe Phe Glu Thr Ile Glu Gly
              740   745   750

Gln Pro Ser Asn Ser Lys Thr Ala Leu Thr Tyr Glu Asp Val Pro Ser
              755   760   765

Ala Pro Pro Asp Asn Ile Gln Ile Gly Met Tyr Asn Gln Thr Ala Gly
          770   775   780

Trp Val Arg Trp Thr Pro Pro Pro Ser Gln His His Asn Gly Asn Leu
785   790   795   800

Tyr Gly Tyr Lys Ile Glu Val Ser Ala Gly Asn Thr Met Lys Val Leu
              805   810   815

Ala Asn Met Thr Leu Asn Ala Thr Thr Thr Ser Val Leu Leu Asn Asn
              820   825   830

Leu Thr Thr Gly Ala Val Tyr Ser Val Arg Leu Asn Ser Phe Thr Lys
          835   840   845

Ala Gly Asp Gly Pro Tyr Ser Lys Pro Ile Ser Leu Phe Met Asp Pro
          850   855   860

6

SUBSTITUTE SHEET (RULE 26)

Thr His His Val His Pro Pro Arg Ala His Pro Ser Gly Thr His Asp
865   870   875   880

Gly Arg His Glu Gly Gln Asp Leu Thr Tyr His Asn Asn Gly Asn Ile
                 885   890   895

Pro Pro Gly Asp Ile Asn Pro Thr Thr His Lys Lys Thr Thr Asp Tyr
             900   905   910

Leu Ser Gly Pro Trp Leu Met Val Leu Val Cys Ile Val Leu Leu Val
             915   920   925

Leu Val Ile Ser Ala Ala Ile Ser Met Val Tyr Phe Lys Arg Lys His
         930   935   940

Gln Met Thr Lys Glu Leu Gly His Leu Ser Val Val Ser Asp Asn Glu
945   950   955   960

Ile Thr Ala Leu Asn Ile Asn Ser Lys Glu Ser Leu Trp Ile Asp His
             965   970   975

His Arg Gly Trp Arg Thr Ala Asp Thr Asp Lys Asp Ser Gly Leu Ser
             980   985   990

Glu Ser Lys Leu Leu Ser His Val Asn Ser Ser Gln Ser Asn Tyr Asn
         995   1000   1005

Asn Ser Asp Gly Gly Thr Asp Tyr Ala Glu Val Asp Thr Arg Asn Leu
         1010   1015   1020

Thr Thr Phe Tyr Asn Cys Arg Lys Ser Pro Asp Asn Pro Thr Pro Tyr
1025   1030   1035   1040

Ala Thr Thr Met Ile Ile Gly Thr Ser Ser Ser Glu Thr Cys Thr Lys
             1045   1050   1055

Thr Thr Ser Ile Ser Ala Asp Lys Asp Ser Gly Thr His Ser Pro Tyr
             1060   1065   1070

Ser Asp Ala Phe Ala Gly Gln Val Pro Ala Val Pro Val Val Lys Ser
             1075   1080   1085

Asn Tyr Leu Gln Tyr Pro Val Glu Pro Ile Asn Trp Ser Glu Phe Leu
         1090   1095   1100

Pro Pro Pro Pro Glu His Pro Pro Pro Ser Ser Thr Tyr Gly Tyr Ala
1105   1110   1115   1120

Gln Gly Ser Pro Glu Ser Ser Arg Lys Ser Ser Lys Ser Ala Gly Ser
             1125   1130   1135

Gly Ile Ser Thr Asn Gln Ser Ile Leu Asn Ala Ser Ile His Ser Ser
             1140   1145   1150

Ser Ser Gly Gly Phe Ser Ala Trp Gly Val Ser Pro Gln Tyr Ala Val
         1155   1160   1165

7

SUBSTITUTE SHEET (RULE 26)

```
Ala Cys Pro Pro Glu Asn Val Tyr Ser Asn Pro Leu Ser Ala Val Ala
     1170                1175                1180
Gly Gly Thr Gln Asn Arg Tyr Gln Ile Thr Pro Thr Asn Gln His Pro
1185                1190                1195                1200
Pro Gln Leu Pro Ala Tyr Phe Ala Thr Thr Gly Pro Gly Gly Ala Val
              1205                1210                1215
Pro Pro Asn His Leu Pro Phe Ala Thr Gln Arg His Ala Ala Ser Glu
              1220                1225                1230
Tyr Gln Ala Gly Leu Asn Ala Ala Arg Cys Ala Gln Ser Arg Ala Cys
          1235                1240                1245
Asn Ser Cys Asp Ala Leu Ala Thr Pro Ser Pro Met Gln Pro Pro Pro
     1250                1255                1260
Pro Val Pro Val Pro Glu Gly Trp Tyr Gln Pro Val His Pro Asn Ser
1265                1270                1275                1280
His Pro Met His Pro Thr Ser Ser Asn His Gln Ile Tyr Gln Cys Ser
              1285                1290                1295
Ser Glu Cys Ser Asp His Ser Arg Ser Ser Gln Ser His Lys Arg Gln
          1300                1305                1310
Leu Gln Leu Glu Glu His Gly Ser Ser Ala Lys Gln Arg Gly Gly His
     1315                1320                1325
His Arg Arg Arg Ala Pro Val Val Gln Pro Cys Met Glu Ser Glu Asn
     1330                1335                1340
Glu Asn Met Leu Ala Glu Tyr Glu Gln Arg Gln Tyr Thr Ser Asp Cys
1345                1350                1355                1360
Cys Asn Ser Ser Arg Glu Gly Asp Thr Cys Ser Cys Ser Glu Gly Ser
              1365                1370                1375
Cys Leu Tyr Ala Glu Ala Gly Glu Pro Ala Pro Arg Gln Met Thr Ala
          1380                1385                1390
Lys Asn Thr
     1395
```

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 4146 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: double

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

8

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

```
GGTGAAAATC CACGCATCAT CGAGCATCCC ATGGACACGA CGGTGCCAAA AAATGATCCA      60
TTTACGTTTA ATTGCCAGGC CGAGGGCAAT CCAACACCAA CCATTCAATG GTTTAAGGAC     120
GGTCGCGAAC TGAAGACGGA TACGGGTTCG CATCGCATAA TGCTGCCCGC CGGGGGTCTA     180
TTCTTTCTCA AGGTTATCCA CTCACGTAGA GAGAGCGATG CGGGCACTTA CTGGTGCGAG     240
GCCAAAAACG AGTTTGGAGT GGCACGGTCC AGGAATGCAA CGTTGCAAGT GGCAGTTCTC     300
CGCGACGAAT TCCGTTTGGA GCCGGCAAAT ACCCGCGTGG CCCAAGGCGA GGTGGCCCTG     360
ATGGAATGCG GTGCCCCCCG AGGATCTCCG GAGCCGCAAA TCTCGTGGCG CAAGAACGGC     420
CAGACCCTGA ATCTTGTCGG GAACAAGCGG ATTCGCATTG TCGACGGTGG CAATCTGGCC     480
ATCCAGGAAG CCCGCCAATC GGACGACGGA CGCTACCAGT GTGTGGTCAA GAATGTGGTT     540
GGCACCCGGG AGTCGGCCAC CGCTTTTCTT AAAGTGCATG TACGTCCATT CCTCATCCGA     600
GGACCCCAGA ATCAGACGGC GGTGGTGGGC AGCTCGGTGG TCTTCCAGTG CCGCATCGGA     660
GGCGATCCCC TGCCTGATGT CCTGTGGCGA CGCACTGCCT CCGGCGGCAA TATGCCACTG     720
CGTAAGTTTT CTTGGCTTCA TTCAGCTTCA GGTCGTGTGC ACGTACTTGA GGACCGCAGT     780
CTGAAGCTGG ACGACGTTAC TCTGGAGGAC ATGGGCGAGT ACACTTGCGA GGCGGACAAT     840
GCGGTGGGCG GCATCACGGC CACTGGCATC CTCACCGTTC ACGCTCCCCC CAAATTTGTG     900
ATACGCCCCA AGAATCAGCT GGTGGAGATC GGTGATGAAG TGCTGTTCGA GTGCCAAGCG     960
AATGGACATC CCCGACCAAC GCTCTACTGG TCGGTGGAGG GCAACAGCTC CCTGCTGCTC    1020
CCCGGCTATC GGGATGGCCG CATGGAAGTG ACCCTGACGC CCGAGGGGCG CTCGGTGCTC    1080
TCGATAGCTC GATTTGCCCG TGAGGATTCC GGAAAGGTGG TCACTTGCAA CGCCCTGAAC    1140
GCCGTGGGCA GCGTCAGCAG TCGGACTGTG GTCAGTGTGG ATACGCAATT CGAGCTGCCA    1200
CCGCCGATTA TCGAACAGGG GCCCGTGAAT CAAACGTTGC CCGTTAAATC AATTGTGGTT    1260
CTGCCATGCC GAACTCTGGG CACTCCAGTG CCACAGGTCT CTTGGTACCT GGATGGCATA    1320
CCCATCGATG TGCAGGAGCA CGAGCGGCGG AATCTTTCGG ACGCTGGAGC CTTAACCATT    1380
TCGGATCTTC AGCGCCACGA GGATGAAGGC TTGTACACCT GCGTGGCCAG CAATCGCAAC    1440
GGAAAATCCT CTTGGAGTGG TTACCTTCGT CTGGACACCC CGACAAATCC GAATATCAAG    1500
TTCTTCAGAG CCCCAGAACT TTCCACCTAC CCAGGGCCGC CAGGAAAACC GCAAATGGTG    1560
GAGAAGGGCG AAAATTCGGT GACTCTCAGC TGGACGAGGA GCAACAAGGT GGGCGGCTCC    1620
AGTCTGGTGG GCTATGTAAT CGAGATGTTT GGCAAAAACG AAACGGATGG CTGGGTGGCT    1680
GTGGGCACTA GGGTGCAAAA TACCACGTTT ACCCAAACGG GTCTGCTGCC GGGTGTGAAT    1740
TACTTCTTTC TAATTCGAGC CGAGAACTCC CATGGCTTAT CACTGCCCAG TCCGATGTCG    1800
GAACCCATTA CGGTGGGAAC GCGCTACTTC AATAGTGGTC TGGATCTGAG CGAGGCTCGT    1860
GCCAGTCTGC TGTCCGGAGA TGTTGTGGAG CTGAGCAACG CCAGTGTGGT GGACTCCACT    1920
AGCATGAAAC TCACCTGGCA GATCATCAAT GGCAAATACG TCGAGGGCTT CTATGTCTAT    1980
GCGAGACAGT TGCCAAATCC AATAGTCAAC AATCCGGCGC CCGTTACTAG CAATACCAAT    2040
CCGCTGCTGG GCTCTACATC CACATCCGCA TCCGCATCCG CCTCGGCATC GGCATTGATT    2100
TCGACAAAGC CAAATATTGC AGCTGCCGGC AAACGTGATG GGGAGACAAA CCAGAGTGGA    2160
GGAGGAGCTC CGACCCCACT GAACACCAAG TATCGCATGC TAACGATTCT CAATGGCGGT    2220
```

SUBSTITUTE SHEET (RULE 26)

```
GGCGCCTCAT CCTGCACCAT CACCGGGCTC GTCCAGTACA CGCTGTATGA ATTTTTCATC    2280
GTGCCATTTT ACAAATCCGT CGAGGGCAAG CCGTCGAATT CGCGCATCGC TCGCACCCTT    2340
GAAGATGTTC CCTCTGAGGC ACCATATGGA ATGGAGGCTC TGCTGTTGAA CTCCTCCGCG    2400
GTCTTCCTCA AATGGAAGGC ACCAGAACTC AAGGATCGGC ATGGTGTTCT CTTGAACTAT    2460
CATGTTATAG TCCGAGGTAT TGACACTGCC CACAATTTCT CACGCATTTT GACAAATGTC    2520
ACCATCGATG CCGCTTCGCC TACTCTGGTT TTGGCCAATC TCACCGAAGG CGTCATGTAC    2580
ACCGTGGGCG TGGCGGCCGG AAATAACGCT GGAGTTGGTC CTTATTGTGT CCCAGCTACT    2640
TTGCGTTTGG ATCCCATCAC AAAGCGACTC GATCCGTTCA TCAATCAGCG GGACCATGTT    2700
AACGATGTGC TGACGCAGCC CTGGTTCATA ATACTCCTGG GCGCCATCCT GGCCGTTCTT    2760
ATGCTGTCCT TTGGCGCAAT GGTCTTTGTG AAGCGCAAGC ACATGATGAT GAAGCAGTCG    2820
GCCCTAAATA CAATGCGTGG CAATCACACG AGCGACGTGC TCAAAATGCC GAGTCTATCG    2880
GCGCGCAATG GAAACGGCTA CTGGCTGGAC TCCTCCACCG GCGGAATGGT GTGGCGTCCC    2940
TCGCCCGGCG GCGACTCGCT GGAGATGCAA AAGGATCACA TCGCCGACTA TGCGCCGGTC    3000
TGCGGTGCCC CCGGTTCTCC GGCCGGCGGT GGCACCTCTT CCGGTGGATC CGGTGGCGCG    3060
GGCAGCGGTG CCAGCGGCGG CGATGACATT CATGGAGGAC ACGGCAGCGA ACGCAATCAG    3120
CAGCGGTACG TGGGCGAGTA CTCCAACATA CCGACCGACT ATGCAGAGGT GTCCAGTTTT    3180
GGCAAGGCAC CCAGCGAGTA TGGTCGGCAT GGCAACGCCT CCCCGGCCCC TTATGCCACC    3240
TCTTCGATCC TGAGTCCCCA CCAGCAGCAA CAGCAGCAGC AGCCGCGTTA TCAACAGCGA    3300
CCAGTGCCCG GCTATGGGCT CCAGCGCCCA ATGCACCCAC ACTACCAGCA GCAGCAGCAT    3360
CAGCAGCAAC AGGCGCAGCA GACGCACCAG CAACACCAGG CTCTCCAGCA GCACCAGCAA    3420
CTGCCACCCA GCAACATCTA CCAGCAGATG TCCACCACCA GCGAGATATA CCCCACGAAC    3480
ACGGGTCCTT CGCGCTCTGT CTACTCTGAG CAGTATTACT ACCCCAAGGA CAAGCAGAGA    3540
CACATCCACA TCACCGAGAA CAAGCTGAGC AACTGCCACA CCTATGAGGC GGCTCCTGGC    3600
GCCAAGCAGT CCTCGCCGAT ATCCTCGCAG TTCGCCAGCG TGAGGCGGCA GCAGCTGCCG    3660
CCCAACTGCA GCATCGGCAG GGAAAGTGCC CGCTTCAAGG TGCTAAACAC GGATCAGGGC    3720
AAGAACCAGC AGAATCTCCT GGATCTCGAC GGCTCCTCGA TGTGCTACAA CGGTCTGGCA    3780
GACTCGGGCT GCGGTGGATC TCCCTCCCCG ATGGCCATGC TGATGTCGCA CGAGGACGAG    3840
CACGCGCTGT ACCACACGGC GGATGGGGAT CTGGACGACA TGGAACGACT GTACGTCAAG    3900
GTGGACGAGC AGCAGCCTCC ACAGCAGCAG CAGCAGCTGA TTCCCCTGGT CCCACAGCAT    3960
CCGGCGGAAG GTCACCTGCA GTCCTGGCGG AATCAGAGCA CGCGGAGCAG TCGGAAGAAC    4020
GGCCAGGAAT GCATCAAGGA ACCCAGCGAG TTGATCTACG CTCCGGGAAG CGTGGCCAGC    4080
GAACGGAGCC TCCTCAGCAA CTCGGGTAGC GGCACCAGCA GCCAGCCAGC TGGCCACAAT    4140
GTCTGA                                                               4146
```

(2) INFORMATION FOR SEQ ID NO:4:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1381 amino acids
        (B) TYPE: amino acid

10

SUBSTITUTE SHEET (RULE 26)

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Gly Glu Asn Pro Arg Ile Ile Glu His Pro Met Asp Thr Thr Val Pro
1   5   10   15

Lys Asn Asp Pro Phe Thr Phe Asn Cys Gln Ala Glu Gly Asn Pro Thr
                20   25   30

Pro Thr Ile Gln Trp Phe Lys Asp Gly Arg Glu Leu Lys Thr Asp Thr
            35   40   45

Gly Ser His Arg Ile Met Leu Pro Ala Gly Gly Leu Phe Phe Leu Lys
        50   55   60

Val Ile His Ser Arg Arg Glu Ser Asp Ala Gly Thr Tyr Trp Cys Glu
65   70   75   80

Ala Lys Asn Glu Phe Gly Val Ala Arg Ser Arg Asn Ala Thr Leu Gln
                85   90   95

Val Ala Val Leu Arg Asp Glu Phe Arg Leu Glu Pro Ala Asn Thr Arg
                100   105   110

Val Ala Gln Gly Glu Val Ala Leu Met Glu Cys Gly Ala Pro Arg Gly
            115   120   125

Ser Pro Glu Pro Gln Ile Ser Trp Arg Lys Asn Gly Gln Thr Leu Asn
        130   135   140

Leu Val Gly Asn Lys Arg Ile Arg Ile Val Asp Gly Gly Asn Leu Ala
145   150   155   160

Ile Gln Glu Ala Arg Gln Ser Asp Asp Gly Arg Tyr Gln Cys Val Val
                165   170   175

Lys Asn Val Val Gly Thr Arg Glu Ser Ala Thr Ala Phe Leu Lys Val
                180   185   190

His Val Arg Pro Phe Leu Ile Arg Gly Pro Gln Asn Gln Thr Ala Val
            195   200   205

Val Gly Ser Ser Val Val Phe Gln Cys Arg Ile Gly Gly Asp Pro Leu
        210   215   220

Pro Asp Val Leu Trp Arg Arg Thr Ala Ser Gly Gly Asn Met Pro Leu
225   230   235   240

Arg Lys Phe Ser Trp Leu His Ser Ala Ser Gly Arg Val His Val Leu
                245   250   255

Glu Asp Arg Ser Leu Lys Leu Asp Asp Val Thr Leu Glu Asp Met Gly
            260   265   270

11

SUBSTITUTE SHEET (RULE 26)

```
Glu Tyr Thr Cys Glu Ala Asp Asn Ala Val Gly Gly Ile Thr Ala Thr
         275              280              285
Gly Ile Leu Thr Val His Ala Pro Pro Lys Phe Val Ile Arg Pro Lys
    290              295              300
Asn Gln Leu Val Glu Ile Gly Asp Glu Val Leu Phe Glu Cys Gln Ala
305              310              315              320
Asn Gly His Pro Arg Pro Thr Leu Tyr Trp Ser Val Glu Gly Asn Ser
              325              330              335
Ser Leu Leu Leu Pro Gly Tyr Arg Asp Gly Arg Met Glu Val Thr Leu
              340              345              350
Thr Pro Glu Gly Arg Ser Val Leu Ser Ile Ala Arg Phe Ala Arg Glu
         355              360              365
Asp Ser Gly Lys Val Val Thr Cys Asn Ala Leu Asn Ala Val Gly Ser
    370              375              380
Val Ser Ser Arg Thr Val Val Ser Val Asp Thr Gln Phe Glu Leu Pro
385              390              395              400
Pro Pro Ile Ile Glu Gln Gly Pro Val Asn Gln Thr Leu Pro Val Lys
              405              410              415
Ser Ile Val Val Leu Pro Cys Arg Thr Leu Gly Thr Pro Val Pro Gln
              420              425              430
Val Ser Trp Tyr Leu Asp Gly Ile Pro Ile Asp Val Gln Glu His Glu
         435              440              445
Arg Arg Asn Leu Ser Asp Ala Gly Ala Leu Thr Ile Ser Asp Leu Gln
    450              455              460
Arg His Glu Asp Glu Gly Leu Tyr Thr Cys Val Ala Ser Asn Arg Asn
465              470              475              480
Gly Lys Ser Ser Trp Ser Gly Tyr Leu Arg Leu Asp Thr Pro Thr Asn
              485              490              495
Pro Asn Ile Lys Phe Phe Arg Ala Pro Glu Leu Ser Thr Tyr Pro Gly
              500              505              510
Pro Pro Gly Lys Pro Gln Met Val Glu Lys Gly Glu Asn Ser Val Thr
         515              520              525
Leu Ser Trp Thr Arg Ser Asn Lys Val Gly Gly Ser Ser Leu Val Gly
    530              535              540
Tyr Val Ile Glu Met Phe Gly Lys Asn Glu Thr Asp Gly Trp Val Ala
545              550              555              560
Val Gly Thr Arg Val Gln Asn Thr Thr Phe Thr Gln Thr Gly Leu Leu
              565              570              575
```

12

SUBSTITUTE SHEET (RULE 26)

```
Pro Gly Val Asn Tyr Phe Phe Leu Ile Arg Ala Glu Asn Ser His Gly
            580                 585                 590

Leu Ser Leu Pro Ser Pro Met Ser Glu Pro Ile Thr Val Gly Thr Arg
            595                 600                 605

Tyr Phe Asn Ser Gly Leu Asp Leu Ser Glu Ala Arg Ala Ser Leu Leu
    610                 615                 620

Ser Gly Asp Val Val Glu Leu Ser Asn Ala Ser Val Val Asp Ser Thr
625                 630                 635                 640

Ser Met Lys Leu Thr Trp Gln Ile Ile Asn Gly Lys Tyr Val Glu Gly
            645                 650                 655

Phe Tyr Val Tyr Ala Arg Gln Leu Pro Asn Pro Ile Val Asn Asn Pro
            660                 665                 670

Ala Pro Val Thr Ser Asn Thr Asn Pro Leu Leu Gly Ser Thr Ser Thr
            675                 680                 685

Ser Ala Ser Ala Ser Ala Ser Ala Ser Ala Leu Ile Ser Thr Lys Pro
    690                 695                 700

Asn Ile Ala Ala Ala Gly Lys Arg Asp Gly Glu Thr Asn Gln Ser Gly
705                 710                 715                 720

Gly Gly Ala Pro Thr Pro Leu Asn Thr Lys Tyr Arg Met Leu Thr Ile
            725                 730                 735

Leu Asn Gly Gly Gly Ala Ser Ser Cys Thr Ile Thr Gly Leu Val Gln
            740                 745                 750

Tyr Thr Leu Tyr Glu Phe Phe Ile Val Pro Phe Tyr Lys Ser Val Glu
            755                 760                 765

Gly Lys Pro Ser Asn Ser Arg Ile Ala Arg Thr Leu Glu Asp Val Pro
    770                 775                 780

Ser Glu Ala Pro Tyr Gly Met Glu Ala Leu Leu Leu Asn Ser Ser Ala
785                 790                 795                 800

Val Phe Leu Lys Trp Lys Ala Pro Glu Leu Lys Asp Arg His Gly Val
            805                 810                 815

Leu Leu Asn Tyr His Val Ile Val Arg Gly Ile Asp Thr Ala His Asn
            820                 825                 830

Phe Ser Arg Ile Leu Thr Asn Val Thr Ile Asp Ala Ala Ser Pro Thr
    835                 840                 845

Leu Val Leu Ala Asn Leu Thr Glu Gly Val Met Tyr Thr Val Gly Val
    850                 855                 860

Ala Ala Gly Asn Asn Ala Gly Val Gly Pro Tyr Cys Val Pro Ala Thr
865                 870                 875                 880
```

13

```
Leu Arg Leu Asp Pro Ile Thr Lys Arg Leu Asp Pro Phe Ile Asn Gln
            885                 890                 895
Arg Asp His Val Asn Asp Val Leu Thr Gln Pro Trp Phe Ile Ile Leu
            900                 905                 910
Leu Gly Ala Ile Leu Ala Val Leu Met Leu Ser Phe Gly Ala Met Val
            915                 920                 925
Phe Val Lys Arg Lys His Met Met Met Lys Gln Ser Ala Leu Asn Thr
        930                 935                 940
Met Arg Gly Asn His Thr Ser Asp Val Leu Lys Met Pro Ser Leu Ser
945                 950                 955                 960
Ala Arg Asn Gly Asn Gly Tyr Trp Leu Asp Ser Ser Thr Gly Gly Met
            965                 970                 975
Val Trp Arg Pro Ser Pro Gly Gly Asp Ser Leu Glu Met Gln Lys Asp
            980                 985                 990
His Ile Ala Asp Tyr Ala Pro Val Cys Gly Ala Pro Gly Ser Pro Ala
            995                 1000                1005
Gly Gly Gly Thr Ser Ser Gly Gly Ser Gly Gly Ala Gly Ser Gly Ala
        1010                1015                1020
Ser Gly Gly Asp Asp Ile His Gly Gly His Gly Ser Glu Arg Asn Gln
1025                1030                1035                1040
Gln Arg Tyr Val Gly Glu Tyr Ser Asn Ile Pro Thr Asp Tyr Ala Glu
            1045                1050                1055
Val Ser Ser Phe Gly Lys Ala Pro Ser Glu Tyr Gly Arg His Gly Asn
            1060                1065                1070
Ala Ser Pro Ala Pro Tyr Ala Thr Ser Ser Ile Leu Ser Pro His Gln
        1075                1080                1085
Gln Gln Gln Gln Gln Gln Pro Arg Tyr Gln Gln Arg Pro Val Pro Gly
        1090                1095                1100
Tyr Gly Leu Gln Arg Pro Met His Pro His Tyr Gln Gln Gln Gln His
1105                1110                1115                1120
Gln Gln Gln Gln Ala Gln Gln Thr His Gln Gln His Gln Ala Leu Gln
            1125                1130                1135
Gln His Gln Gln Leu Pro Pro Ser Asn Ile Tyr Gln Gln Met Ser Thr
        1140                1145                1150
Thr Ser Glu Ile Tyr Pro Thr Asn Thr Gly Pro Ser Arg Ser Val Tyr
        1155                1160                1165
Ser Glu Gln Tyr Tyr Tyr Pro Lys Asp Lys Gln Arg His Ile His Ile
        1170                1175                1180                Thr
```

14

```
Glu Asn Lys Leu Ser Asn Cys His Thr Tyr Glu Ala Ala Pro Gly
    1185            1190            1195            1200
    Ala Lys Gln Ser Ser Pro Ile Ser Ser Gln Phe Ala Ser Val Arg Arg
                1205            1210            1215
    Gln Gln Leu Pro Pro Asn Cys Ser Ile Gly Arg Glu Ser Ala Arg Phe
                1220            1225            1230
    Lys Val Leu Asn Thr Asp Gln Gly Lys Asn Gln Gln Asn Leu Leu Asp
            1235            1240            1245
    Leu Asp Gly Ser Ser Met Cys Tyr Asn Gly Leu Ala Asp Ser Gly Cys
        1250            1255            1260
    Gly Gly Ser Pro Ser Pro Met Ala Met Leu Met Ser His Glu Asp Glu
    1265            1270             1275            1280
    His Ala Leu Tyr His Thr Ala Asp Gly Asp Leu Asp Asp Met Glu Arg
                1285            1290            1295
    Leu Tyr Val Lys Val Asp Glu Gln Gln Pro Pro Gln Gln Gln Gln Gln
                1300            1305            1310
    Leu Ile Pro Leu Val Pro Gln His Pro Ala Glu Gly His Leu Gln Ser
            1315            1320            1325
    Trp Arg Asn Gln Ser Thr Arg Ser Ser Arg Lys Asn Gly Gln Glu Cys
        1330            1335            1340
    Ile Lys Glu Pro Ser Glu Leu Ile Tyr Ala Pro Gly Ser Val Ala Ser
    1345            1350            1355            1360
    Glu Arg Ser Leu Leu Ser Asn Ser Gly Ser Gly Thr Ser Ser Gln Pro
                1365            1370            1375
    Ala Gly His Asn Val
                1380
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 3894 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: double

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

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

```
ATGTACTATC TAGGTTTTTA CCACACTCAC ACACACACAC ACACATACAT AAATTTTGAT      60
AAAATTCCTA ATGCCTCAAA TCTCGCTCCC GTGATAATCG AACATCCCAT CGATGTGGTG     120
GTATCTAGGG GATCGCCAGC AACCCTCAAC TGTGGTGCAA AGCCATCTAC CGCCAAAATC     180
```

15

```
ACATGGTACA AGGATGGACA GCCCGTAATC ACGAATAAGG AGCAAGTGAA CAGCCACCGG    240
ATTGTTCTCG ACACGGGATC CCTGTTTCTT CTGAAAGTGA ATAGTGGAAA AAACGGAAAA    300
GACAGCGATG CGGGAGCGTA CTATTGTGTG GCCAGCAACG AGCACGGAGA AGTGAAGTCG    360
AACGAAGGAT CGTTAAAATT GGCGATGCTT CGCGAAGACT TTCGAGTTCG GCCAAGAACA    420
GTTCAGGCTC TTGGTGGAGA GATGGCCGTT CTGGAATGCA GTCCGCCACG TGGATTCCCG    480
GAGCCGGTTG TGAGCTGGCG GAAAGACGAC AAAGAGCTCC GAATTCAAGA CATGCCACGA    540
TACACTCTAC ACTCTGACGG AAACCTCATC ATTGATCCGG TCGATCGAAG CGATTCTGGT    600
ACTTATCAGT GTGTTGCCAA CAACATGGTC GGAGAACGGG TGTCCAATCC CGCAAGATTG    660
AGTGTCTTTG AGAAACCAAA GTTTGAGCAA GAACCCAAGG ACATGACGGT CGACGTCGGA    720
GCCGCAGTGC TGTTTGATTG TCGTGTGACT GGAGATCCTC AACCACAAAT TACGTGGAAA    780
CGCAAAAATG AGCCGATGCC AGTTACACGT GCATACATTG CCAAGGATAA TCGGGGGTTG    840
AGAATCGAAA GAGTTCAACC ATCAGACGAA GGTGAATACG TTTGCTATGC ACGAAATCCA    900
GCGGGAACTC TTGAAGCATC TGCACATCTT CGTGTCCAGG CACCTCCATC CTTCCAGACA    960
AAACCAGCAG ACCAGTCAGT TCCAGCTGGA GGCACGGCAA CTTTTGAATG CACCTTGGTC   1020
GGTCAACCGA GTCCCGCCTA TTTTTGGAGC AAGGAAGGCC AACAGGATCT TCTTTTCCCA   1080
AGTTATGTGT CCGCTGATGG TAGAACGAAA GTTTCACCAA CTGGAACATT GACAATTGAG   1140
GAAGTTCGTC AAGTTGATGA GGGAGCTTAT GTGTGCGCTG GAATGAACTC GGCAGGAAGC   1200
TCGTTGAGCA AGGCAGCTTT GAAAGCAACA TTTGAAACCA AAGGCCGTGT CCAAAAAAAA   1260
AAGAGCAAAA TGGGCAAACA GAAACAAAAA AATGTTCAAT CAATTATCAA ATATTTAATT   1320
TCAGCCGTGA CCGGAAACAC ACCCGCCAAA CCACCACCAA CAATCGAGCA TGGTCATCAA   1380
AATCAGACCC TTATGGTTGG ATCATCAGCC ATCCTTCCAT GTCAGGCTAG CGGAAAACCA   1440
ACTCCAGGAA TATCATGGCT CAGGGATGGG CTACCTATTG ACATTACAGA TAGTCGTATC   1500
AGTCAACATT CAACGGGAAG TCTACATATT GCCGATTTAA AGAAACCTGA CACCGGAGTT   1560
TACACTTGCA TTGCGAAGAA CGAGGATGGA GAGTCAACAT GGTCGGCATC TCTGACTGTT   1620
GAAGATCACA CTAGCAATGC ACAATTTGTT CGGATGCCGG ATCCATCGAA CTTCCCGTCT   1680
TCTCCAACGC AACCCATTAT TGTCAATGTC ACTGATACCG AAGTAGAGCT CCACTGGAAT   1740
GCTCCCTCCA CATCTGGCGC AGGACCAATC ACTGGTTATA TCATTCAGTA CTACAGTCCA   1800
GACCTCGGAC AGACGTGGTT TAACATTCCA GACTACGTGG CATCTACTGA ATATAGAATA   1860
AAGGGTCTGA AACCATCTCA CTCGTATATG TTTGTGATTC GAGCAGAAAA TGAGAAAGGT   1920
ATTGGAACGC CGAGTGTGTC GTCGGCTCTC GTTACCACTA GCAAGCCAGC AGCTCAAGTT   1980
GCGCTTTCTG ACAAGAACAA AATGGACATG GCCATCGCTG AGAAGAGACT CACTTCGGAA   2040
CAACTCATAA AACTCGAGGA AGTGAAGACT ATTAATTCTA CGGCCGTTCG TTTGTTCTGG   2100
AAGAAGAGGA AACTTGAAGA GCTGATTGAT GGTTACTACA TCAAGTGGAG AGGGCCTCCA   2160
AGAACCAATG ATAATCAATA CGTGAATGTG ACCAGCCCTA GCACCGAAAA CTATGTTGTT   2220
TCAAATTTAA TGCCATTCAC CAACTATGAG TTTTTCGTGA TTCCTTATCA TTCCGGAGTT   2280
CATAGTATTC ATGGAGCACC GAGTAATTCC ATGGACGTGT TGACCGCCGA AGCTCCACCT   2340
TCATTGCCAC CAGAGGATGT GCGAATCCGT ATGCTCAACC TGACCACTCT TCGTATCTCT   2400
TGGAAAGCAC CAAAAGCCGA CGGCATCAAC GGAATTCTCA AAGGATTCCA AATTGTTATT   2460
```

16

```
GTTGGTCAAG CGCCCAACAA CAATCGGAAC ATCACTACAA ACGAGAGAGC TGCCAGTGTT    2520

ACTCTGTTCC ATTTAGTGAC TGGAATGACG TATAAAATTC GTGTAGCGGC TAGAAGCAAT    2580

GGTGGAGTTG GAGTCTCACA TGGAACGAGT GAAGTCATCA TGAATCAAGA CACGCTGGAA    2640

AAACACCTTG CTGCTCAACA AGAAAACGAA TCATTTTTGT ATGGGCTGAT CAATAAATCT    2700

CATGTTCCTG TGATTGTCAT TGTTGCAATT CTGATTATTT TCGTAGTCAT CATTATAGCC    2760

TATTGTTACT GGAGGAATAG CAGAAACAGT GATGGAAAGG ATCGAAGTTT TATAAAGATC    2820

AATGATGGAA GTGTTCATAT GGCTTCGAAT AATCTTTGGG ATGTTGCACA AAATCCGAAT    2880

CAGAATCCAA TGTACAACAC TGCTGGAAGA ATGACTATGA ACAATAGAAA TGGCCAGGCT    2940

CTCTATTCGC TGACACCAAA TGCGCAAGAC TTTTTCAACA ATTGTGATGA CTACAGTGGA    3000

ACGATGCACA GACCAGGATC CGAGCATCAC TATCATTATG CTCAACTGAC TGGCGGACCT    3060

GGTAATGCGA TGTCTACTTT TTATGGAAAC CAATATCACG ATGATCCATC TCCATATGCC    3120

ACCACAACAC TGGTCCTGTC GAACCAACAA CCAGCTTGGC TCAATGACAA AATGCTTCGC    3180

GCGCCAGCAA TGCCAACAAA TCCCGTGCCA CCAGAGCCAC CGGCGCGATA TGCAGATCAT    3240

ACCGCTGGAA GACGATCTCG ATCGAGCCGT GCATCCGATG GGAGAGGAAC TCTGAATGGC    3300

GGACTCCATC ACCGGACTAG CGGAAGTCAA CGGTCGGATA GTCCACCTCA CACAGATGTG    3360

AGCTATGTTC AGCTTCACTC ATCCGATGGA ACTGGTAGTA GTAAGGAAAG AACTGGGGAG    3420

CGGAGAACAC CACCGAATAA GACTCTGATG GACTTTATTC CGCCACCACC TTCCAATCCA    3480

CCACCACCTG GAGGGCACGT TTATGACACA GCAACTAGGC GTCAGTTGAA TCGTGGAAGT    3540

ACTCCACGAG AAGACACCTA CGATTCGGTC AGTGACGGAG CTTTTGCTCG GGTTGATGTG    3600

AATGCAAGGC CAACGAGTCG GAATCGGAAT TTGGGAGGAA GGCCGCTGAA AGGGAAACGA    3660

GACGACGATA GTCAGCGGTC TTCGTTGATG ATGGACGATG ATGGTGGATC TTCTGAAGCT    3720

GACGGGGAGA ACTCTGAAGG AGACGTTCCG CGTGGAGGTG TTAGAAAAGC AGTTCCTCGA    3780

ATGGGTATCT CTGCAAGTAC GCTGGCTCAT AGTTGTTACG GGACAAACGG CACTGCTCAA    3840

CGATTCCGGT CAATTCCACG TAACAATGGA ATCGTCACAC AAGAACAAAC TTGA          3894
```

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1297 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

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

```
Met Tyr Tyr Leu Gly Phe Tyr His Thr His Thr His Thr His Thr Tyr
1               5                   10                  15

Ile Asn Phe Asp Lys Ile Pro Asn Ala Ser Asn Leu Ala Pro Val Ile
                20                  25                  30

Ile Glu His Pro Ile Asp Val Val Val Ser Arg Gly Ser Pro Ala Thr
```

17

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

18

SUBSTITUTE SHEET (RULE 26)

```
                    340                    345                    350
        Gly Gln Gln Asp Leu Leu Phe Pro Ser Tyr Val Ser Ala Asp Gly Arg
                355                    360                    365
        Thr Lys Val Ser Pro Thr Gly Thr Leu Thr Ile Glu Glu Val Arg Gln
            370                    375                    380
        Val Asp Glu Gly Ala Tyr Val Cys Ala Gly Met Asn Ser Ala Gly Ser
        385                    390                    395                    400
        Ser Leu Ser Lys Ala Ala Leu Lys Ala Thr Phe Glu Thr Lys Gly Arg
                        405                    410                    415
        Val Gln Lys Lys Lys Ser Lys Met Gly Lys Gln Lys Gln Lys Asn Val
                    420                    425                    430
        Gln Ser Ile Ile Lys Tyr Leu Ile Ser Ala Val Thr Gly Asn Thr Pro
                435                    440                    445
        Ala Lys Pro Pro Pro Thr Ile Glu His Gly His Gln Asn Gln Thr Leu
            450                    455                    460
        Met Val Gly Ser Ser Ala Ile Leu Pro Cys Gln Ala Ser Gly Lys Pro
        465                    470                    475                    480
        Thr Pro Gly Ile Ser Trp Leu Arg Asp Gly Leu Pro Ile Asp Ile Thr
                        485                    490                    495
        Asp Ser Arg Ile Ser Gln His Ser Thr Gly Ser Leu His Ile Ala Asp
                    500                    505                    510
        Leu Lys Lys Pro Asp Thr Gly Val Tyr Thr Cys Ile Ala Lys Asn Glu
                515                    520                    525
        Asp Gly Glu Ser Thr Trp Ser Ala Ser Leu Thr Val Glu Asp His Thr
                530                    535                    540
        Ser Asn Ala Gln Phe Val Arg Met Pro Asp Pro Ser Asn Phe Pro Ser
        545                    550                    555                    560
        Ser Pro Thr Gln Pro Ile Ile Val Asn Val Thr Asp Thr Glu Val Glu
                        565                    570                    575
        Leu His Trp Asn Ala Pro Ser Thr Ser Gly Ala Gly Pro Ile Thr Gly
                580                    585                    590
        Tyr Ile Ile Gln Tyr Tyr Ser Pro Asp Leu Gly Gln Thr Trp Phe Asn
                595                    600                    605
        Ile Pro Asp Tyr Val Ala Ser Thr Glu Tyr Arg Ile Lys Gly Leu Lys
            610                    615                    620
        Pro Ser His Ser Tyr Met Phe Val Ile Arg Ala Glu Asn Glu Lys Gly
        625                    630                    635                    640
        Ile Gly Thr Pro Ser Val Ser Ser Ala Leu Val Thr Thr Ser Lys Pro
```

19

SUBSTITUTE SHEET (RULE 26)

```
                       645                     650                     655
     Ala Ala Gln Val Ala Leu Ser Asp Lys Asn Lys Met Asp Met Ala Ile
               660                     665                     670
     Ala Glu Lys Arg Leu Thr Ser Glu Gln Leu Ile Lys Leu Glu Glu Val
               675                     680                     685
     Lys Thr Ile Asn Ser Thr Ala Val Arg Leu Phe Trp Lys Lys Arg Lys
           690                     695                     700
     Leu Glu Glu Leu Ile Asp Gly Tyr Tyr Ile Lys Trp Arg Gly Pro Pro
     705                     710                     715                     720
     Arg Thr Asn Asp Asn Gln Tyr Val Asn Val Thr Ser Pro Ser Thr Glu
                       725                     730                     735
     Asn Tyr Val Val Ser Asn Leu Met Pro Phe Thr Asn Tyr Glu Phe Phe
                   740                     745                     750
     Val Ile Pro Tyr His Ser Gly Val His Ser Ile His Gly Ala Pro Ser
               755                     760                     765
     Asn Ser Met Asp Val Leu Thr Ala Glu Ala Pro Pro Ser Leu Pro Pro
           770                     775                     780
     Glu Asp Val Arg Ile Arg Met Leu Asn Leu Thr Thr Leu Arg Ile Ser
     785                     790                     795                     800
     Trp Lys Ala Pro Lys Ala Asp Gly Ile Asn Gly Ile Leu Lys Gly Phe
                   805                     810                     815
     Gln Ile Val Ile Val Gly Gln Ala Pro Asn Asn Asn Arg Asn Ile Thr
               820                     825                     830
     Thr Asn Glu Arg Ala Ala Ser Val Thr Leu Phe His Leu Val Thr Gly
               835                     840                     845
     Met Thr Tyr Lys Ile Arg Val Ala Ala Arg Ser Asn Gly Gly Val Gly
           850                     855                     860
     Val Ser His Gly Thr Ser Glu Val Ile Met Asn Gln Asp Thr Leu Glu
     865                     870                     875                     880
     Lys His Leu Ala Ala Gln Gln Glu Asn Glu Ser Phe Leu Tyr Gly Leu
               885                     890                     895
     Ile Asn Lys Ser His Val Pro Val Ile Val Ile Val Ala Ile Leu Ile
               900                     905                     910
     Ile Phe Val Val Ile Ile Ile Ala Tyr Cys Tyr Trp Arg Asn Ser Arg
               915                     920                     925
     Asn Ser Asp Gly Lys Asp Arg Ser Phe Ile Lys Ile Asn Asp Gly Ser
           930                     935                     940
     Val His Met Ala Ser Asn Asn Leu Trp Asp Val Ala Gln Asn Pro Asn
```

20

SUBSTITUTE SHEET (RULE 26)

```
945                    950                    955                    960
Gln Asn Pro Met Tyr Asn Thr Ala Gly Arg Met Thr Met Asn Asn Arg
                  965                    970                    975
Asn Gly Gln Ala Leu Tyr Ser Leu Thr Pro Asn Ala Gln Asp Phe Phe
              980                    985                    990
Asn Asn Cys Asp Asp Tyr Ser Gly Thr Met His Arg Pro Gly Ser Glu
          995                    1000                   1005
His His Tyr His Tyr Ala Gln Leu Thr Gly Gly Pro Gly Asn Ala Met
      1010                   1015                   1020
Ser Thr Phe Tyr Gly Asn Gln Tyr His Asp Asp Pro Ser Pro Tyr Ala
1025                   1030                   1035                   1040
Thr Thr Thr Leu Val Leu Ser Asn Gln Gln Pro Ala Trp Leu Asn Asp
                  1045                   1050                   1055
Lys Met Leu Arg Ala Pro Ala Met Pro Thr Asn Pro Val Pro Pro Glu
              1060                   1065                   1070
Pro Pro Ala Arg Tyr Ala Asp His Thr Ala Gly Arg Arg Ser Arg Ser
          1075                   1080                   1085
Ser Arg Ala Ser Asp Gly Arg Gly Thr Leu Asn Gly Gly Leu His His
      1090                   1095                   1100
Arg Thr Ser Gly Ser Gln Arg Ser Asp Ser Pro Pro His Thr Asp Val
1105                   1110                   1115                   1120
Ser Tyr Val Gln Leu His Ser Ser Asp Gly Thr Gly Ser Ser Lys Glu
                  1125                   1130                   1135
Arg Thr Gly Glu Arg Arg Thr Pro Pro Asn Lys Thr Leu Met Asp Phe
              1140                   1145                   1150
Ile Pro Pro Pro Ser Asn Pro Pro Pro Gly Gly His Val Tyr
          1155                   1160                   1165
Asp Thr Ala Thr Arg Arg Gln Leu Asn Arg Gly Ser Thr Pro Arg Glu
      1170                   1175                   1180
Asp Thr Tyr Asp Ser Val Ser Asp Gly Ala Phe Ala Arg Val Asp Val
1185                   1190                   1195                   1200
Asn Ala Arg Pro Thr Ser Arg Asn Arg Asn Leu Gly Gly Arg Pro Leu
              1205                   1210                   1215
Lys Gly Lys Arg Asp Asp Asp Ser Gln Arg Ser Ser Leu Met Met Asp
          1220                   1225                   1230
Asp Asp Gly Gly Ser Ser Glu Ala Asp Gly Glu Asn Ser Glu Gly Asp
      1235                   1240                   1245
Val Pro Arg Gly Gly Val Arg Lys Ala Val Pro Arg Met Gly Ile Ser
```

21

SUBSTITUTE SHEET (RULE 26)

```
            1250              1255              1260
    Ala Ser Thr Leu Ala His Ser Cys Tyr Gly Thr Asn Gly Thr Ala Gln
    1265              1270              1275              1280
    Arg Phe Arg Ser Ile Pro Arg Asn Asn Gly Ile Val Thr Gln Glu Gln
                      1285              1290              1295
    Thr
```

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 4956 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: double

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

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

```
ATGAAATGGA AACATGTTCC TTTTTTGGTC ATGATATCAC TCCTCAGCTT ATCCCCAAAT      60
CACCTGTTTC TGGCCCAGCT TATTCCAGAC CCTGAAGATG TAGAGAGGGG GAACGACCAC     120
GGGACGCCAA TCCCCACCTC TGATAACGAT GACAATTCGC TGGGCTATAC AGGCTCCCGT     180
CTTCGTCAGG AAGATTTTCC ACCTCGCATT GTTGAACACC CTTCAGACCT GATTGTCTCA     240
AAAGGAGAAC CTGCAACTTT GAACTGCAAA GCTGAAGGCC GCCCCACACC CACTATTGAA     300
TGGTACAAAG GGGGAGAGAG AGTGGAGACA GACAAAGATG ACCCTCGCTC ACACCGAATG     360
TTGCTGCCGA GTGGATCTTT ATTTTTCTTA CGTATAGTAC ATGGACGGAA AAGTAGACCT     420
GATGAAGGAG TCTATGTCTG TGTAGCAAGG AATTACCTTG GAGAGGCTGT GAGCCACAAT     480
GCATCGCTGG AAGTAGCCAT ACTTCGGGAT GACTTCAGAC AAAACCCTTC GGATGTCATG     540
GTTGCAGTAG GAGAGCCTGC AGTAATGGAA TGCCAACCTC CACGAGGCCA TCCTGAGCCC     600
ACCATTTCAT GGAAGAAAGA TGGCTCTCCA CTGGATGATA AGATGAAAG AATAACTATA     660
CGAGGAGGAA AGCTCATGAT CACTTACACC CGTAAAAGTG ACGCTGGCAA ATATGTTTGT     720
GTTGGTACCA ATATGGTTGG GGAACGTGAG AGTGAAGTAG CCGAGCTGAC TGTCTTAGAG     780
AGACCATCAT TTGTGAAGAG ACCCAGTAAC TTGGCAGTAA CTGTGGATGA CAGTGCAGAA     840
TTTAAATGTG AGGCCCGAGG TGACCCTGTA CCTACAGTAC GATGGAGGAA AGATGATGGA     900
GAGCTGCCCA AATCCAGATA TGAAATCCGA GATGATCATA CCTTGAAAAT TAGGAAGGTG     960
ACAGCTGGTG ACATGGGTTC ATACACTTGT GTTGCAGAAA ATATGGTGGG CAAAGCTGAA    1020
GCATCTGCTA CTCTGACTGT TCAAGAACCT CCACATTTTG TTGTGAAACC CCGTGACCAG    1080
GTTGTTGCTT TGGGACGGAC TGTAACTTTT CAGTGTGAAG CAACCGGAAA TCCTCAACCA    1140
GCTATTTTCT GGAGGAGAGA AGGGAGTCAG AATCTACTTT TCTCATATCA ACCACCACAG    1200
TCATCCAGCC GATTTTCAGT CTCCCAGACT GGCGACCTCA CAATTACTAA TGTCCAGCGA    1260
TCTGATGTTG GTTATTACAT CTGCCAGACT TTAAATGTTG CTGGAAGCAT CATCACAAAG    1320
GCATATTTGG AAGTTACAGA TGTGATTGCA GATCGGCCTC CCCCAGTTAT TCGACAAGGT    1380
```

```
CCTGTGAATC AGACTGTAGC CGTGGATGGC ACTTTCGTCC TCAGCTGTGT GGCCACAGGC    1440

AGTCCAGTGC CCACCATTCT GTGGAGAAAG GATGGAGTCC TCGTTTCAAC CCAAGACTCT    1500

CGAATCAAAC AGTTGGAGAA TGGAGTACTG CAGATCCGAT ATGCTAAGCT GGGTGATACT    1560

GGTCGGTACA CCTGCATTGC ATCAACCCCC AGTGGTGAAG CAACATGGAG TGCTTACATT    1620

GAAGTTCAAG AATTTGGAGT TCCAGTTCAG CCTCCAAGAC CTACTGACCC AAATTTAATC    1680

CCTAGTGCCC CATCAAAACC TGAAGTGACA GATGTCAGCA GAAATACAGT CACATTATCG    1740

TGGCAACCAA ATTTGAATTC AGGAGCAACT CCAACATCTT ATATTATAGA AGCCTTCAGC    1800

CATGCATCTG GTAGCAGCTG GCAGACCGTA GCAGAGAATG TGAAAACAGA AACATCTGCC    1860

ATTAAAGGAC TCAAACCTAA TGCAATTTAC CTTTTCCTTG TGAGGGCAGC TAATGCATAT    1920

GGAATTAGTG ATCCAAGCCA AATATCAGAT CCAGTGAAAA CACAAGATGT CCTACCAACA    1980

AGTCAGGGGG TGGACCACAA GCAGGTCCAG AGAGAGCTGG GAAATGCTGT TCTGCACCTC    2040

CACAACCCCA CCGTCCTTTC TTCCTCTTCC ATCGAAGTGC ACTGGACAGT AGATCAACAG    2100

TCTCAGTATA TACAAGGATA TAAAATTCTC TATCGGCCAT CTGGAGCCAA CCACGGAGAA    2160

TCAGACTGGT TAGTTTTTGA AGTGAGGACG CCAGCCAAAA ACAGTGTGGT AATCCCTGAT    2220

CTCAGAAAGG GAGTCAACTA TGAAATTAAG GCTCGCCCTT TTTTTAATGA ATTTCAAGGA    2280

GCAGATAGTG AAATCAAGTT TGCCAAAACC CTGGAAGAAG CACCCAGTGC CCCACCCCAA    2340

GGTGTAACTG TATCCAAGAA TGATGGAAAC GGAACTGCAA TTCTAGTTAG TTGGCAGCCA    2400

CCTCCAGAAG ACACTCAAAA TGGAATGGTC CAAGAGTATA AGGTTTGGTG TCTGGGCAAT    2460

GAAACTCGAT ACCACATCAA CAAAACAGTG GATGGTTCCA CCTTTTCCGT GGTCATTCCC    2520

TTTCTTGTTC CTGGAATCCG ATACAGTGTG GAAGTGGCAG CCAGCACTGG GGCTGGGTCT    2580

GGGGTAAAGA GTGAGCCTCA GTTCATCCAG CTGGATGCCC ATGGAAACCC TGTGTCACCT    2640

GAGGACCAAG TCAGCCTCGC TCAGCAGATT TCAGATGTGG TGAAGCAGCC GGCCTTCATA    2700

GCAGGTATTG GAGCAGCCTG TTGGATCATC CTCATGGTCT TCAGCATCTG GCTTTATCGA    2760

CACCGCAAGA AGAGAAACGG ACTTACTAGT ACCTACGCGG GTATCAGAAA AGTCCCGTCT    2820

TTTACCTTCA CACCAACAGT AACTTACCAG AGAGGAGGCG AAGCTGTCAG CAGTGGAGGG    2880

AGGCCTGGAC TTCTCAACAT CAGTGAACCT GCCGCGCAGC CATGGCTGGC AGACACGTGG    2940

CCTAATACTG GCAACAACCA CAATGACTGC TCCATCAGCT GCTGCACGGC AGGCAATGGA    3000

AACAGCGACA GCAACCTCAC TACCTACAGT CGCCCAGCTG ATTGTATAGC AAATTATAAC    3060

AACCAACTGG ATAACAAACA AACAAATCTG ATGCTCCCTG AGTCAACTGT TTATGGTGAT    3120

GTGGACCTTA GTAACAAAAT CAATGAGATG AAAACCTTCA ATAGCCCAAA TCTGAAGGAT    3180

GGGCGTTTTG TCAATCCATC AGGGCAGCCT ACTCCTTACG CCACCACTCA GCTCATCCAG    3240

TCAAACCTCA GCAACAACAT GAACAATGGC AGCGGGGACT CTGGCGAGAA GCACTGGAAA    3300

CCACTGGGAC AGCAGAAACA AGAAGTGGCA CCAGTTCAGT ACAACATCGT GGAGCAAAAC    3360

AAGCTGAACA AAGATTATCG AGCAAATGAC ACAGTTCCTC CAACTATCCC ATACAACCAA    3420

TCATACGACC AGAACACAGG AGGATCCTAC AACAGCTCAG ACCGGGGCAG TAGTACATCT    3480

GGGAGTCAGG GGCACAAGAA AGGGGCAAGA ACACCCAAGG TACCAAAACA GGGTGGCATG    3540

AACTGGGCAG ACCTGCTTCC TCCTCCCCCA GCACATCCTC CTCCACACAG CAATAGCGAA    3600

GAGTACAACA TTTCTGTAGA TGAAAGCTAT GACCAAGAAA TGCCATGTCC CGTGCCACCA    3660
```

23

SUBSTITUTE SHEET (RULE 26)

```
GCAAGGATGT ATTTGCAACA AGATGAATTA GAAGAGGAGG AAGATGAACG AGGCCCCACT    3720
CCCCCTGTTC GGGGAGCAGC TTCTTCTCCA GCTGCCGTGT CCTATAGCCA TCAGTCCACT    3780
GCCACTCTGA CTCCCTCCCC ACAGGAAGAA CTCCAGCCCA TGTTACAGGA TTGTCCAGAG    3840
GAGACTGGCC ACATGCAGCA CCAGCCCGAC AGGAGACGGC AGCCTGTGAG TCCTCCTCCA    3900
CCACCACGGC CGATCTCCCC TCCACATACC TATGGCTACA TTTCAGGACC CCTGGTCTCA    3960
GATATGGATA CGGATGCGCC AGAAGAGGAA GAAGACGAAG CCGACATGGA GGTAGCCAAG    4020
ATGCAAACCA GAAGGCTTTT GTTACGTGGG CTTGAGCAGA CACCTGCCTC CAGTGTTGGG    4080
GACCTGGAGA GCTCTGTCAC GGGGTCCATG ATCAACGGCT GGGGCTCAGC CTCAGAGGAG    4140
GACAACATTT CCAGCGGACG CTCCAGTGTT AGTTCTTCGG ACGGCTCCTT TTTCACTGAT    4200
GCTGACTTTG CCCAGGCAGT CGCAGCAGCG GCAGAGTATG CTGGTCTGAA AGTAGCACGA    4260
CGGCAAATGC AGGATGCTGC TGGCCGTCGA CATTTTCATG CGTCTCAGTG CCCTAGGCCC    4320
ACAAGTCCCG TGTCTACAGA CAGCAACATG AGTGCCGCCG TAATGCAGAA AACCAGACCA    4380
GCCAAGAAAC TGAAACACCA GCCAGGACAT CTGCGCAGAG AAACCTACAC AGATGATCTT    4440
CCACCACCTC CTGTGCCGCC ACCTGCTATA AAGTCACCTA CTGCCCAATC CAAGACACAG    4500
CTGGAAGTAC GACCTGTAGT GGTGCCAAAA CTCCCTTCTA TGGATGCAAG AACAGACAGA    4560
TCATCAGACA GAAAAGGAAG CAGTTACAAG GGGAGAGAAG TGTTGGATGG AAGACAGGTT    4620
GTTGACATGC GAACAAATCC AGGTGATCCC AGAGAAGCAC AGGAACAGCA AAATGACGGG    4680
AAAGGACGTG GAAACAAGGC AGCAAAACGA GACCTTCCAC CAGCAAAGAC TCATCTCATC    4740
CAAGAGGATA TTCTACCTTA TTGTAGACCT ACTTTTCCAA CATCAAATAA TCCCAGAGAT    4800
CCCAGTTCCT CAAGCTCAAT GTCATCAAGA GGATCAGGAA GCAGACAAAG AGAACAAGCA    4860
AATGTAGGTC GAAGAAATAT TGCAGAAATG CAGGTACTTG GAGGATATGA AAGAGGAGAA    4920
GATAATAATG AAGAATTAGA GGAAACTGAA AGCTGA                              4956
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1651 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

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

```
Met Lys Trp Lys His Val Pro Phe Leu Val Met Ile Ser Leu Leu Ser
1               5                   10                  15
Leu Ser Pro Asn His Leu Phe Leu Ala Gln Leu Ile Pro Asp Pro Glu
                20                  25                  30
Asp Val Glu Arg Gly Asn Asp His Gly Thr Pro Ile Pro Thr Ser Asp
            35                  40                  45
Asn Asp Asp Asn Ser Leu Gly Tyr Thr Gly Ser Arg Leu Arg Gln Glu
```

24

SUBSTITUTE SHEET (RULE 26)

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

25

SUBSTITUTE SHEET (RULE 26)

```
          355                    360                    365
Thr Phe Gln Cys Glu Ala Thr Gly Asn Pro Gln Pro Ala Ile Phe Trp
    370                    375                    380
Arg Arg Glu Gly Ser Gln Asn Leu Leu Phe Ser Tyr Gln Pro Pro Gln
385                    390                    395                    400
Ser Ser Ser Arg Phe Ser Val Ser Gln Thr Gly Asp Leu Thr Ile Thr
                   405                    410                    415
Asn Val Gln Arg Ser Asp Val Gly Tyr Tyr Ile Cys Gln Thr Leu Asn
                   420                    425                    430
Val Ala Gly Ser Ile Ile Thr Lys Ala Tyr Leu Glu Val Thr Asp Val
         435                    440                    445
Ile Ala Asp Arg Pro Pro Pro Val Ile Arg Gln Gly Pro Val Asn Gln
    450                    455                    460
Thr Val Ala Val Asp Gly Thr Phe Val Leu Ser Cys Val Ala Thr Gly
465                    470                    475                    480
Ser Pro Val Pro Thr Ile Leu Trp Arg Lys Asp Gly Val Leu Val Ser
                   485                    490                    495
Thr Gln Asp Ser Arg Ile Lys Gln Leu Glu Asn Gly Val Leu Gln Ile
                   500                    505                    510
Arg Tyr Ala Lys Leu Gly Asp Thr Gly Arg Tyr Thr Cys Ile Ala Ser
         515                    520                    525
Thr Pro Ser Gly Glu Ala Thr Trp Ser Ala Tyr Ile Glu Val Gln Glu
    530                    535                    540
Phe Gly Val Pro Val Gln Pro Pro Arg Pro Thr Asp Pro Asn Leu Ile
545                    550                    555                    560
Pro Ser Ala Pro Ser Lys Pro Glu Val Thr Asp Val Ser Arg Asn Thr
                   565                    570                    575
Val Thr Leu Ser Trp Gln Pro Asn Leu Asn Ser Gly Ala Thr Pro Thr
                   580                    585                    590
Ser Tyr Ile Ile Glu Ala Phe Ser His Ala Ser Gly Ser Ser Trp Gln
         595                    600                    605
Thr Val Ala Glu Asn Val Lys Thr Glu Thr Ser Ala Ile Lys Gly Leu
    610                    615                    620
Lys Pro Asn Ala Ile Tyr Leu Phe Leu Val Arg Ala Ala Asn Ala Tyr
625                    630                    635                    640
Gly Ile Ser Asp Pro Ser Gln Ile Ser Asp Pro Val Lys Thr Gln Asp
                   645                    650                    655
Val Leu Pro Thr Ser Gln Gly Val Asp His Lys Gln Val Gln Arg Glu
```

26

SUBSTITUTE SHEET (RULE 26)

```
             660                    665                    670
Leu Gly Asn Ala Val Leu His Leu His Asn Pro Thr Val Leu Ser Ser
         675                    680                    685
Ser Ser Ile Glu Val His Trp Thr Val Asp Gln Gln Ser Gln Tyr Ile
     690                    695                    700
Gln Gly Tyr Lys Ile Leu Tyr Arg Pro Ser Gly Ala Asn His Gly Glu
705                    710                    715                    720
Ser Asp Trp Leu Val Phe Glu Val Arg Thr Pro Ala Lys Asn Ser Val
                 725                    730                    735
Val Ile Pro Asp Leu Arg Lys Gly Val Asn Tyr Glu Ile Lys Ala Arg
             740                    745                    750
Pro Phe Phe Asn Glu Phe Gln Gly Ala Asp Ser Glu Ile Lys Phe Ala
         755                    760                    765
Lys Thr Leu Glu Glu Ala Pro Ser Ala Pro Pro Gln Gly Val Thr Val
     770                    775                    780
Ser Lys Asn Asp Gly Asn Gly Thr Ala Ile Leu Val Ser Trp Gln Pro
785                    790                    795                    800
Pro Pro Glu Asp Thr Gln Asn Gly Met Val Gln Glu Tyr Lys Val Trp
                 805                    810                    815
Cys Leu Gly Asn Glu Thr Arg Tyr His Ile Asn Lys Thr Val Asp Gly
             820                    825                    830
Ser Thr Phe Ser Val Val Ile Pro Phe Leu Val Pro Gly Ile Arg Tyr
         835                    840                    845
Ser Val Glu Val Ala Ala Ser Thr Gly Ala Gly Ser Gly Val Lys Ser
     850                    855                    860
Glu Pro Gln Phe Ile Gln Leu Asp Ala His Gly Asn Pro Val Ser Pro
865                    870                    875                    880
Glu Asp Gln Val Ser Leu Ala·Gln Gln Ile Ser Asp Val Val Lys Gln
                 885                    890                    895
Pro Ala Phe Ile Ala Gly Ile Gly Ala Ala Cys Trp Ile Ile Leu Met
             900                    905                    910
Val Phe Ser Ile Trp Leu Tyr Arg His Arg Lys Lys Arg Asn Gly Leu
         915                    920                    925
Thr Ser Thr Tyr Ala Gly Ile Arg Lys Val Pro Ser Phe Thr Phe Thr
     930                    935                    940
Pro Thr Val Thr Tyr Gln Arg Gly Gly Glu Ala Val Ser Ser Gly Gly
945                    950                    955                    960
Arg Pro Gly Leu Leu Asn Ile Ser Glu Pro Ala Ala Gln Pro Trp Leu
```

27

```
                    965                970                975
       Ala Asp Thr Trp Pro Asn Thr Gly Asn Asn His Asn Asp Cys Ser Ile
                980                985                990
       Ser Cys Cys Thr Ala Gly Asn Gly Asn Ser Asp Ser Asn Leu Thr Thr
            995                1000                1005
       Tyr Ser Arg Pro Ala Asp Cys Ile Ala Asn Tyr Asn Asn Gln Leu Asp
         1010                1015                1020
       Asn Lys Gln Thr Asn Leu Met Leu Pro Glu Ser Thr Val Tyr Gly Asp
       1025                1030                1035                1040
       Val Asp Leu Ser Asn Lys Ile Asn Glu Met Lys Thr Phe Asn Ser Pro
                1045                1050                1055
       Asn Leu Lys Asp Gly Arg Phe Val Asn Pro Ser Gly Gln Pro Thr Pro
                1060                1065                1070
       Tyr Ala Thr Thr G Ile Gln Ser Asn Leu Ser Asn Asn Met Asn
                1075                1080                1085
       Asn Gly Ser Gly Asp Ser Gly Glu Lys His Trp Lys Pro Leu Gly Gln
            1090                1095                1100
       Gln Lys Gln Glu Val Ala Pro Val Gln Tyr Asn Ile Val Glu Gln Asn
       1105                1110                1115                1120
       Lys Leu Asn Lys Asp Tyr Arg Ala Asn Asp Thr Val Pro Pro Thr Ile
                1125                1130                1135
       Pro Tyr Asn Gln Ser Tyr Asp Gln Asn Thr Gly Gly Ser Tyr Asn Ser
                1140                1145                1150
       Ser Asp Arg Gly Ser Ser Thr Ser Gly Ser Gln Gly His Lys Lys Gly
            1155                1160                1165
       Ala Arg Thr Pro Lys Val Pro Lys Gln Gly Gly Met Asn Trp Ala Asp
            1170                1175                1180
       Leu Leu Pro Pro Pro Pro Ala His Pro Pro Pro His Ser Asn Ser Glu
       1185                1190                1195                1200
       Glu Tyr Asn Ile Ser Val Asp Glu Ser Tyr Asp Gln Glu Met Pro Cys
                1205                1210                1215
       Pro Val Pro Pro Ala Arg Met Tyr Leu Gln Gln Asp Glu Leu Glu Glu
                1220                1225                1230
       Glu Glu Asp Glu Arg Gly Pro Thr Pro Pro Val Arg Gly Ala Ala Ser
            1235                1240                1245
       Ser Pro Ala Ala Val Ser Tyr Ser His Gln Ser Thr Ala Thr Leu Thr
            1250                1255                1260
       Pro Ser Pro Gln Glu Glu Leu Gln Pro Met Leu Gln Asp Cys Pro Glu
```

28

SUBSTITUTE SHEET (RULE 26)

```
    1265                   1270                   1275                   1280
Glu Thr Gly His Met Gln His Gln Pro Asp Arg Arg Arg Gln Pro Val
                    1285                   1290                   1295
Ser Pro Pro Pro Pro Pro Arg Pro Ile Ser Pro Pro His Thr Tyr Gly
                1300                   1305                   1310
Tyr Ile Ser Gly Pro Leu Val Ser Asp Met Asp Thr Asp Ala Pro Glu
            1315                   1320                   1325
Glu Glu Glu Asp Glu Ala Asp Met Glu Val Ala Lys Met Gln Thr Arg
        1330                   1335                   1340
Arg Leu Leu Leu Arg Gly Leu Glu Gln Thr Pro Ala Ser Ser Val Gly
    1345                   1350                   1355                   1360
Asp Leu Glu Ser Ser Val Thr Gly Ser Met Ile Asn Gly Trp Gly Ser
                1365                   1370                   1375
Ala Ser Glu Glu Asp Asn Ile Ser Ser Gly Arg Ser Ser Val Ser Ser
            1380                   1385                   1390
Ser Asp Gly Ser Phe Phe Thr Asp Ala Asp Phe Ala Gln Ala Val Ala
        1395                   1400                   1405
Ala Ala Ala Glu Tyr Ala Gly Leu Lys Val Ala Arg Arg Gln Met Gln
    1410                   1415                   1420
Asp Ala Ala Gly Arg Arg His Phe His Ala Ser Gln Cys Pro Arg Pro
1425                   1430                   1435                   1440
Thr Ser Pro Val Ser Thr Asp Ser Asn Met Ser Ala Ala Val Met Gln
                1445                   1450                   1455
Lys Thr Arg Pro Ala Lys Lys Leu Lys His Gln Pro Gly His Leu Arg
            1460                   1465                   1470
Arg Glu Thr Tyr Thr Asp Asp Leu Pro Pro Pro Pro Val Pro Pro Pro
        1475                   1480                   1485
Ala Ile Lys Ser Pro Thr Ala Gln Ser Lys Thr Gln Leu Glu Val Arg
    1490                   1495                   1500
Pro Val Val Val Pro Lys Leu Pro Ser Met Asp Ala Arg Thr Asp Arg
1505                   1510                   1515                   1520
Ser Ser Asp Arg Lys Gly Ser Ser Tyr Lys Gly Arg Glu Val Leu Asp
                1525                   1530                   1535
Gly Arg Gln Val Val Asp Met Arg Thr Asn Pro Gly Asp Pro Arg Glu
            1540                   1545                   1550
Ala Gln Glu Gln Gln Asn Asp Gly Lys Gly Arg Gly Asn Lys Ala Ala
        1555                   1560                   1565
Lys Arg Asp Leu Pro Pro Ala Lys Thr His Leu Ile Gln Glu Asp Ile
```

SUBSTITUTE SHEET (RULE 26)

```
        1570              1575              1580
  Leu Pro Tyr Cys Arg Pro Thr Phe Pro Thr Ser Asn Asn Pro Arg Asp
  1585              1590              1595              1600
  Pro Ser Ser Ser Ser Ser Met Ser Ser Arg Gly Ser Gly Ser Arg Gln
                    1605              1610              1615
  Arg Glu Gln Ala Asn Val Gly Arg Arg Asn Ile Ala Glu Met Gln Val
                    1620              1625              1630
  Leu Gly Gly Tyr Glu Arg Gly Glu Asp Asn Asn Glu Glu Leu Glu Glu
              1635              1640              1645
  Thr Glu Ser
        1650
```

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1300 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: double

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (ix) FEATURE:

        (A) NAME/KEY: misc_feature

        (B) LOCATION: 855..1187

        (D) OTHER INFORMATION: /note= "N signifies gap in sequence"

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

```
CAGATTGTTG CTCAAGGTCG AACAGTGACA TTTCCCTGTG AAACTAAAGG AAACCCACAG      60
CCAGCTGTTT TTTGGCAGAA AGAAGGCAGC CAGAACCTAC TTTTCCCAAA CCAACCCCAG     120
CAGCCCAACA GTAGATGCTC AGTGTCACCA ACTGGAGACC TCACAATCAC CAACATTCAA     180
CGTTCCGACG CGGGTTACTA CATCTGCCAG GCTTTAACTG TGGCAGGAAG CATTTTAGCA     240
AAAGCTCAAC TGGAGGTTAC TGATGTTTTG ACAGATAGAC CTCCACCTAT AATTCTACAA     300
GGCCCAGCCA ACCAAACGCT GGCAGTGGAT GGTACAGCGT TACTGAAATG TAAAGCCACT     360
GGTGATCCTC TTCCTGTAAT TAGCTGGTTA AAGGAGGGAT TTACTTTTCC GGGTAGAGAT     420
CCAAGAGCAA CAATTCAAGA GCAAGGCACA CTGCAGATTA GAATTTACG GATTTCTGAT     480
ACTGGCACTT ATACTTGTGT GGCTACAAGT TCAAGTGGAG AGGCTTCCTG GAGTGCAGTG     540
CTGGATGTGA CAGAGTCTGG AGCAACAATC AGTAAAAACT ATGATTTAAG TGACCTGCCA     600
GGGCCACCAT CCAAACCGCA AGTCACTGAT GTTACTAAGA CAGTGTCAC CTTGTCCTGG     660
CAGCCAGGTA CCCCTGGAAC CCTTCCAGCA AGTGCATATA TCATTGAGGC TTTCAGCCAA     720
TCAGTGAGCA ACAGCTGGCA GACCGTGGCA AACCATGTAA AGACCACCCT CTATACTGTA     780
AGAGGACTGC GGCCCAATAC AATCTACTTA TTCATGGTCA GAGCGATCAA CCCCAAGGTY     840
```

TCAGTGACCC AAGTNAAACC ACAGAAAAAC AATGGATCCA CTTGGGCCAA TGTCCCTCTA   900

CCTCCCCCCC CAGTCCAGCC CCTTCCTGGC ACGGAGCTGG AACACTATGC AGTGGAACAA   960

CAAGAAAATG GCTATGACAG TGATAGCTGG TGCCCACCAT TGCCAGTACA AACTTACTTA   1020

CACCAAGGTC TGGAAGATGA ACTGGAAGAA GATGATGATA GGGTCCCAAC ACCTCCTGTT   1080

CGAGGCGTGG CTTCTTCTCC TGCTATCTCC TTTGGACAGC AGTCCACTGC AACTCTTACT   1140

CCATCCCCAC GGGAAGAGAT GCAACCCATG CTGCAGGCTT CACCTNTTTA CCTCCTCTCA   1200

AAGACCTCGA CCTACCAGCC CATTTTCTAC TGACAGTAAC ACCAGTGCAG CCCTGAGTCA   1260

AAGTCAGAGG CCTCGGCCCA CTAAAAAACA CAAGGGAGGG   1300

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 434 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (ix) FEATURE:

        (A) NAME/KEY: Modified-site

        (B) LOCATION: 285..396

        (D) OTHER INFORMATION: /note= "Xaa signifies gap in sequence"

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

Gln Ile Val Ala Gln Gly Arg Thr Val Thr Phe Pro Cys Glu Thr Lys

1   5   10   15

Gly Asn Pro Gln Pro Ala Val Phe Trp Gln Lys Glu Gly Ser Gln Asn

        20   25   30

Leu Leu Phe Pro Asn Gln Pro Gln Gln Pro Asn Ser Arg Cys Ser Val

        35   40   45

Ser Pro Thr Gly Asp Leu Thr Ile Thr Asn Ile Gln Arg Ser Asp Ala

        50   55   60

Gly Tyr Tyr Ile Cys Gln Ala Leu Thr Val Ala Gly Ser Ile Leu Ala

65   70   75   80

Lys Ala Gln Leu Glu Val Thr Asp Val Leu Thr Asp Arg Pro Pro Pro

        85   90   95

Ile Ile Leu Gln Gly Pro Ala Asn Gln Thr Leu Ala Val Asp Gly Thr

        100   105   110

Ala Leu Leu Lys Cys Lys Ala Thr Gly Asp Pro Leu Pro Val Ile Ser

        115   120   125

Trp Leu Lys Glu Gly Phe Thr Phe Pro Gly Arg Asp Pro Arg Ala Thr

31

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

32

SUBSTITUTE SHEET (RULE 26)

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 444 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: double

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

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

```
GCCCAGGCAG TTGCTGCAGC TGCGGAGTAT GCGGGCCTGA AAGTGGCTCG CCGCCAAATG        60
CAAGATGCTG CTGGCCGCCG CCACTTCCAT GCCTCTCAGT GCCCAAGGCC CACGAGTCCT       120
GTGTCCACAG ACAGCAACAT GAGTGCTGTT GTGATCCAGA AGCCAGACC CGCCAAGAAG        180
CAGAAACACC AGCCAGGACA TCTGCGCAGG GAAGCCTACG CAGATGATCT TCCACCCCCT       240
CCAGTGCCAC CACCTGCTAT AAAATCGCCC ACTGTCCAGT CCAAGGCACA GCTGGAGGTA       300
CGGCCTGTCA TGGTGCCAAA ACTCGCGTCT ATAGAAGCAA GGACAGATAG ATCGTCAGAC       360
AGAAAAGGAG GCAGTTACAA GGGGAGAGAA GCTCTGGATG GAAGACAAGT CACTGACCTG       420
CGAACAAATC CAAGTGACCC CAGA                                              444
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 148 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

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

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

33

SUBSTITUTE SHEET (RULE 26)

Gln Leu Glu Val Arg Pro Val Met Val Pro Lys Leu Ala Ser Ile Glu
            100   105   110

Ala Arg Thr Asp Arg Ser Ser Asp Arg Lys Gly Gly Ser Tyr Lys Gly
            115   120   125

Arg Glu Ala Leu Asp Gly Arg Gln Val Thr Asp Leu Arg Thr Asn Pro
        130   135   140

Ser Asp Pro Arg
145

34

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/18   C07K14/475   A61K38/18

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12N   C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMEST DATABASE Accession number AA499193 03-JUL-1997 (Rel. 52, Created) Marra M. ET AL. XP002094384	1,8
Y	see the whole document ---	2-7,9
Y	TEAR G ET AL: "To cross or not to cross: a genetic analysis of guidance at the midline." PERSPECT DEV NEUROBIOL, 1993, 1 (4) P183-94, XP002094379 UNITED STATES see page 191, left-hand column, paragraph 2 - page 193, left-hand column, paragraph 2 --- -/--	2-7,9

X	Further documents are listed in the continuation of box C.

| X | Patent family members are listed in annex. |

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 February 1999	09/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

Form PCT/ISA/210 (second sheet) (July 1992)

1

page 1 of 3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EMEST DATABASE Accession number AA263962 21-MAR-1997 (Rel. 51, Created) Harvey D. ET AL. XP002094385 see the whole document	1,8
X	EMEST DATABASE Accession number C68275 20-SEP-1997 (Rel. 52, Created) Kohara Y. ET AL. XP002094386 see the whole document	1,8
X	EMEST DATABASE Accession number H52936 22-SEP-1995 (Rel. 45, Created) Hillier L. ET AL XP002094387 see the whole document	1,8
X	EMEST DATABASE Accession number H19148 02-JUL-1995 (Rel. 44, Created) Hillier L. ET AL. XP002094388 see the whole document	1,8
X	KOLODZIEJ PA ET AL: "FRAZZLED ENCODES A DROSOPHILA MEMBER OF THE DCC IMMUNOGLOBULIN SUBFAMILY AND IS REQUIRED FOR CNS AND MOTOR AXON GUIDANCE" CELL, 1996, 87, 197-204, XP002094380 see the whole document	1-9
X	WO 95 13367 A (UNIV CALIFORNIA ;UNIV COLUMBIA (US)) 18 May 1995 see page 43 - page 54	1-9
A	SEEGER M ET AL: "Mutations affecting growth cone guidance in Drosophila: genes necessary for guidance toward or away from the midline." NEURON, MAR 1993, 10 (3) P409-26, XP002094381 UNITED STATES see abstract see page 419, right-hand column, paragraph 4 - page 425	2-7,9

-/--

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	TEAR G ET AL: "commissureless controls growth cone guidance across the CNS midline in Drosophila and encodes a novel membrane protein." NEURON, MAR 1996, 16 (3) P501-14, XP002094382 UNITED STATES see the whole document	1-9
P,X	KIDD T ET AL: "Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors." CELL, JAN 23 1998, 92 (2) P205-15, XP002094383 UNITED STATES see the whole document	1-8

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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

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

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

Remark: Although claim 9, in as far as it concerns an in vivo method, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compund/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

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

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

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9513367 A	18-05-1995	US 5565331 A	15-10-1996
		CA 2174971 A	18-05-1995
		EP 0804564 A	05-11-1997
		JP 9509305 T	22-09-1997