An isolated and substantially pure form of the SLIT protein and sequence elements thereof, antibodies thereto and diagnostics and therapeutics utilizing such proteins and antibodies. A method for treating neurodegenerative disease, traumatic injury to a neural tissue or affecting the angiogenic process in a patient comprising administering to the patient an effective amount of the SLIT protein.
### FOR THE PURPOSES OF INFORMATION ONLY

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

<table>
<thead>
<tr>
<th>Code</th>
<th>Country</th>
<th>Code</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>Austria</td>
<td>ES</td>
<td>Spain</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
<td>FI</td>
<td>Finland</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>FR</td>
<td>France</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>GA</td>
<td>Gabon</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>GN</td>
<td>Guinea</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>GR</td>
<td>Greece</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>KP</td>
<td>Democratic People's Republic of Korea</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>CI</td>
<td>Côte d'Ivoire</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>CM</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>CS</td>
<td>Czechoslovakia</td>
<td>CS</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>DE+</td>
<td>Germany</td>
<td>DE+</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MN</td>
<td>Mongolia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SN</td>
<td>Senegal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SU+</td>
<td>Soviet Union</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>Chad</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>Togo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US</td>
<td>United States of America</td>
</tr>
</tbody>
</table>

+ Any designation of “SU” has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.
PURIFIED SLIT PROTEIN AND SEQUENCE ELEMENTS THEREOF
GOVERNMENT RIGHTS

This invention was made with United States government support under Grant NS 26084 from the National Institute of Health. The United States government thus has certain rights in this invention.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention concerns an isolated and substantially pure form of the SLIT protein and sequence elements thereof, antibodies thereto and diagnostics and therapeutics utilizing such proteins and antibodies.

Background Information

Proteins containing epidermal growth factor (EGF)-like sequences have been shown to play an important role in many aspects of eukaryotic cell control, acting as signals for proliferation, growth inhibition, and differentiation. A common feature of these proteins is their involvement in extracellular events and ligand-receptor interactions. In characterizing genomic DNA identified by cross-hybridization to the sequence coding for the tandem EGF-repeats of Notch, a gene involved in Drosophila neurogenesis, the isolation and partial characterization of sequences from an unlinked locus that coded for EGF-repeats have previously been reported. This sequence was shown to correspond to the SLIT locus and it was established that null mutations result in disruptions
of the embryonic CNS. (Rothberg, J.M., Hartley, D.A.,
Walther, Z., Artavanis-Tsakonas, S., (1988). slit: An EGF-
Homologous Locus of D. Melanogaster Involved in the
Development of the Embryonic Central Nervous System. Cell 55,
1047-1059).

The involvement of SLIT in cell interaction events is
suggested by the presence of EGF-like repeats in the deduced
protein sequence. Furthermore, both in situ hybridization,
as well as antibody staining of embryos demonstrated that the
highest level of slit expression is restricted to a special
group of six midline glial cells that interact with and later
envrap developing commissural axons. Together, these
findings are of particular interest, given the mutant
phenotype and the evidence that, in both vertebrates and
invertebrates, glial cells participate in neutral outgrowth
through cell-cell contact and the secretion of diffusible
of neuronal growth cones in grasshopper embryo. III.
Recognition of specific glial pathways. J. Neurosci. 6,3542-

The appearance of a glial scaffold in Drosophila before
axonal outgrowth as well as the extension of pioneer growth
cones along the surfaces of these glial cells, suggests that
these glia play an instructive role in the determination of
the major axon pathways in the central nervous system (CNS)
development of axon pathways in the drosophila CNS. I.A glial
scaffold appears before the first growth cones. J. Neurosci.
Embryonic development of axon pathways in the drosophila CNS.
II. Behavior of pioneer growth cones. J. Neurosci. 9, 2402-
2411).
It has long been thought that the extracellular environment influences the regulation of gene expression and the morphogenesis of cells during embryonic development (McDonald, J.A. (1989). Matrix regulation of cell shape and gene expression. Current Opinion in Cell Biology 1, 995-999). In the nervous system, the morphogenetic events accompanying the formation of early structures have been shown to be dependent on the properties of the molecules that form their extracellular environment (see Jessell, (1988) Neuron. 1, 3-13). In vitro and in vivo studies suggest that growth cone guidance and axonal pathway selection are influenced by adhesive interactions between axons and extracellular matrix molecules (Sanes, J.R. (1989). Extracellular matrix molecules that influence neural development. Ann. Rev. Neurosci. 12, 491-516).

Furthermore, specific constituents of the extracellular environment have been shown to affect neurite outgrowth in vitro and have been detected in vivo in the developing central and peripheral nervous systems (see Rutishauser, (1989), Neural cell-to-cell adhesion and recognition Current Opinion in Cell Biology, 1, 898-904).
<table>
<thead>
<tr>
<th>Amino Acid Codes</th>
<th>Single Letter Code</th>
<th>Three Letter Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine</td>
<td>A</td>
<td>Ala</td>
</tr>
<tr>
<td>cysteine</td>
<td>C</td>
<td>Cys</td>
</tr>
<tr>
<td>aspartic acid</td>
<td>D</td>
<td>Asp</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>E</td>
<td>Glu</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>F</td>
<td>Phe</td>
</tr>
<tr>
<td>glycine</td>
<td>G</td>
<td>Gly</td>
</tr>
<tr>
<td>histidine</td>
<td>H</td>
<td>His</td>
</tr>
<tr>
<td>isoleucine</td>
<td>I</td>
<td>Ile</td>
</tr>
<tr>
<td>lysine</td>
<td>K</td>
<td>Lys</td>
</tr>
<tr>
<td>leucine</td>
<td>L</td>
<td>Leu</td>
</tr>
<tr>
<td>methionine</td>
<td>M</td>
<td>Met</td>
</tr>
<tr>
<td>asparagine</td>
<td>N</td>
<td>Asn</td>
</tr>
<tr>
<td>proline</td>
<td>P</td>
<td>Pro</td>
</tr>
<tr>
<td>glutamine</td>
<td>Q</td>
<td>Gln</td>
</tr>
<tr>
<td>arginine</td>
<td>R</td>
<td>Arg</td>
</tr>
<tr>
<td>serine</td>
<td>S</td>
<td>Ser</td>
</tr>
<tr>
<td>threonine</td>
<td>T</td>
<td>Thr</td>
</tr>
<tr>
<td>valine</td>
<td>V</td>
<td>Val</td>
</tr>
<tr>
<td>tryptophan</td>
<td>W</td>
<td>Trp</td>
</tr>
<tr>
<td>tyrosine</td>
<td>Y</td>
<td>Tyr</td>
</tr>
<tr>
<td>any amino acid</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
SUMMARY OF THE INVENTION

The present invention relates to recombinant proteins produced using all or part of the SLIT DNA sequences and exhibiting SLIT-like properties. The invention is also directed to the corresponding recombinant constructs and probes, including, genomic, cDNA, and synthetic DNA and protein sequences, as well as antibodies generated against specific domains of the SLIT protein. The invention also concerns prokaryotic and eukaryotic expression of all or parts of the SLIT-like genes from metazoan organisms, including, but not limited to its Flank-LRR-Flank and epidermal growth factor like sequences.

More specifically, the present invention concerns an isolated and substantially pure form of the SLIT protein comprising SEQ.ID. NO. 2, obtained by recombinant means from SEQ. ID. NO. 1 or from a natural source. The invention also relates to an isolated DNA segment encoding the entire SLIT protein, a recombinant expression vector comprising such DNA segment and a recombinant host microorganism containing a DNA expression vector comprising a DNA sequence consisting essentially of a DNA sequence encoding the entire SLIT protein.
The present invention also concerns a consensus amino flank-LRR-carboxy-flank sequence element of the SLIT protein (SEQ. I.D. NO. 8) comprising
(a) an amino-flanking region comprising the sequence
CPxxCxC....xGxxVDCxxGlx...xαFxαPxDTTx,
(b) a leucine-rich repeat region comprising one or more repeats of the sequence xxxxFxxLxxLxxLxxNxxIxxL, and
(c) a carboxy-flanking region comprising the sequence
P(W or F)xC(D or N)Cxα....W(L or F)xxxxxxxxxxxxx...........RCxx
PxxxxxxzzzzzzzzzzzzzxzPx...C(P or S).

The present invention is also directed to the following four amino-flank-LRR-carboxy-flank sequence elements of the SLIT protein.

(1) A first amino-flank-LRR-carboxy-flank sequence element of the SLIT protein (SEQ. I.D. NO. 3) wherein
(a) the amino-flanking region comprises the sequence:
CPRVCSC TGLNVDCSHRGLT SVPRKISADVER,
(b) the leucine-rich region comprises the sequence:

```text
LELQGLNTVI
YETQFCRLTLMLGLTDSQQTVI
ERUSFODLVTELIDSHNVIITV
GRRVFQGASLQLQDDROITCL
DEHAFKGLVELEIL-LQNNLTLSL
PHNIFGGLGRSLRALRED
```

and

(c) the carboxy-flanking region comprises the sequence
PFACD CHL SWLRSFLRSATRLAPY TCQSPQQLKQNVADLDQEFK
CSGLTEHAPMECGAENS.
(2) A second amino-flank-LRR-carboxy-flank sequence element of the SLIT protein (SEQ. I.D. No. 4) wherein
   (a) the amino-flanking region comprises the sequence:
       CPHPCRC ADGIVDCREKSLT SVPVTLPPDDTD
   (b) the leucine-rich region comprises the sequence:
       VRLEONFTEL
       PFKSFSSFARIDLSGNNISRI
       ANDALSGKQTLTTLVLYGNNIKDL
       PSGVTKGLGSLRQQNLEICSI
       RCKAFLDNLSSLSSLNYGNNQSL
       ANGTDAMKSMKTVHLSN
       and
   (c) the carboxy-flanking region comprises the sequence
       PPICNCNL RWLADYLHKIFETSGARCESPFKRHRRRRLRESLRKFK
       CSWGELRMKLSEGCRMDSD.

(3) A third amino-flank-LRR-carboxy-flank sequence element of the SLIT protein (SEQ. I.D. No. 5) wherein
   (a) the amino-flanking region comprises the sequence:
       CPAMCHC EGTTVDCTGRGLK EIPRDIPLHTTE
   (b) the leucine-rich repeat region comprises the sequence:
       LLND\^_{ELGRIS}
       SDGLFGRFLHLVKLELRRQDQTLGI
       EPNFAEGSHIGELGGERKIKEI
       SNKHFLGHLKATLNLNQNNSCV
       MPDSFELNLSLTSNLASN
       and
   (c) the carboxy-flanking region comprises the sequence:
       PFNCNCNH LWFAECEVKSKSLNGGAA RCGAPSKVQDKLPH SEEK
       CSSENSEGCLGD GY.

(4) A fourth amino-flank-LRR-carboxy-flank sequence element of the SLIT protein (SEQ. I.D. No. 6) wherein
   (a) the amino-flanking region comprising the sequence
       CPPSCTC TGGTVEACSNQQLK EIPRGIPAE
   (b) the leucine-rich repeat region comprises the sequence:
(c) the carboxy-flanking region comprises the sequence:
PLYCQCGL KWPSDWIKLDYVEFGIA RCAEPEQMKLKLSSLTPSSSFRV
CRGRVRNDILAKCNA.

The invention also relates to the alternate splice
segment of the SLIT protein residing at the seventh epidermal
growth factor (EGF) sequence element of the SLIT protein
comprising the sequence GEGSTEPFTVT (SEQ. I.D. NO. 7).

The invention further concerns the carboxy terminal
region of the SLIT protein (SEQ. I.D. NO. 9) residing after
the seventh EGF.

Still further, the present invention is directed to
combinations comprising one or more amino-flank-LRR-carboxy-
flank sequence elements as defined above and one or more EGF-
like repeat elements of the SLIT protein, provided that the
combination does not include the naturally occurring
configuration of the SLIT protein. The aforesaid combination
can include the aforesaid alternative splice segment of the
SLIT protein.

The present invention also encompasses antibodies to the
SLIT protein or to the portions thereof encompassed by the
present invention. Such antibodies are produced when the SLIT
protein as described herein is introduced in an animal, e.g.,
a rabbit, mouse or rat, so as to raise antibodies in the
animal and such antibodies are then withdrawn from the animal.
The present invention is further directed to monoclonal
antibodies to the SLIT protein or to the portions thereof
encompassed by the present invention.
The invention also concerns diagnostics and therapeutics. Immunoassays are provided by the invention. In one such immunoassay a method for detecting the SLIT protein or a shed portion thereof in a bodily fluid from, for example, a human, is provided comprising contacting the bodily fluid with the antibodies to the SLIT protein described herein and detecting for the presence of the SLIT protein. Alternatively, a method of detecting autoimmune antibodies to the SLIT protein or a shed portion thereof in a bodily fluid from, for example, a human, is provided which comprises contacting the bodily fluid with the SLIT protein or portions thereof as defined herein and detecting for the presence of autoimmune antibodies to the SLIT protein.

The invention is also directed to detecting chromosomal rearrangements in the SLIT locus comprising hybridizing a nucleic acid (DNA or RNA) from a patient, e.g., a human patient, with a nucleic acid sequence from the SLIT (genomic) locus and detecting for the level of expression or an aberrant rearrangement.

The invention also relates to a pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile (pharmaceutically acceptable) preparation comprising an effective amount of the SLIT protein as disclosed herein or to a portion thereof in admixture with a pharmaceutically acceptable carrier. The invention further includes the administration of such pharmaceutical preparation or a SLIT protein or a portion thereof, without a carrier, as disclosed herein or a portion thereof encompassed by the present invention in an effective amount to treat patients, e.g., humans, suffering from neurodegenerative disease or a
traumatic injury to a neural tissue or to affect the angiogenic process.

In addition, the invention is also directed to a class of multifunctional "TAGON" molecules which facilitate interactions between cell surface receptors involved in cell regulation and extracellular matrix molecules.

Thus the invention also concerns a protein, TAGON, that allows for the formation of a molecular bridge between axonally associated receptors and extracellular matrix molecules.

The invention also concerns a pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of a TAGON protein in admixture with a pharmaceutically acceptable carrier.

The present invention is also directed to a method for the treatment of a neurodegenerative disease, for treating tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of a TAGON protein, either alone or in admixture with a pharmaceutically acceptable carrier.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1A schematically depicts the SLIT transcript. Fig. 1B is a restriction map of the genomic sequence containing the SLIT transcript.

Fig 2A schematically depicts the SLIT protein. Fig. 2B schematically depicts the elements of the SLIT protein.
Fig. 3 comprises twelve photographs depicting the SLIT message, protein, and promoter activation at three stages of embryogenesis by in situ hybridization, antibody staining and enhancer trap detection.

Fig. 4 comprise three photographs of an embryo undergoing dorsal closure stained with anti-SLIT antibodies.

Fig. 5 is a photograph depicting immunoelectron microscopic localization of SLIT in embryonic CNS to midline cells and axonal tracts.

Fig. 6 depicts immunoblots which show the secretion of SLIT from cultured cells.

Fig. 7 comprises eight photographs which show the pattern of expression of β-galactosidase in MP2 cells and the midline neuroepithelium and its progeny compared in wild type and null mutant embryos.

Fig. 8 comprises six photographs which show that levels of SLIT expression correlate with disruptions of midline cells and axon pathways.

DETAILED DESCRIPTION OF THE DRAWINGS

Fig. 1. Transcription Unit and Molecular Characterization of SLIT P-element Enhancer Trap Alleles
In Fig. 1, the SLIT transcript (Fig. 1A) is shown aligned above the corresponding genomic sequence (Fig. 1B). Transcription is shown from left to right. Alternating light and dark shading patterns are used to represent the five EcoRI restriction fragments in the CDNA with the numbers above indicating their size in base pairs. Where known precisely,
the location of splice sites are shown by a connecting "v". Other exonic regions are shown as blocks aligned approximately with corresponding genomic sequence. The location of primers used to confirm the splice variation in the SLIT transcript and the resulting 33bp alternate segment are indicated by opposing horizontal arrows and a vertical bar, respectively. The location of the primer used to detect the P-element inserts is shown by a left pointing arrow near the 5' end of the transcript. Fig. 1B is a restriction map of the genomic sequence containing the SLIT transcription unit. Labeled triangles indicate the site of insertion of the enhancer trap construct in the various P-element SLIT alleles. Their nucleotide position relative to the consensus transcription initiation site is shown in parenthesis (B=BamHI; E=EcoRI; H=HindIII; S=SalI).

**Fig. 2. Conservation of Flank-LRR-Flank Domains in Known Adhesive Proteins**

Fig. 2A is a schematic representation of the SLIT protein. The putative signal sequence and amino and carboxy-terminal ends of the protein are indicated. The four consecutive Flank-LRR-Flank regions, the 7 EGF repeats and the 11 amino acid connecting segment, the result of differential splicing at the COOH-terminal of the 7th EGF repeat, are shown. Single LRRs have been shown to form β-sheets in solution and, as depicted here, may form anti-parallel sheets (Krantz, D. E., and Zipursky, S. L. (1990). Drosophila chaoptin, a member of the leucine-rich repeat family, is a photoreceptor cell-specific adhesion molecule. EMBO J.9, 1969-1977). Tandem EGF-like repeats in other ECM proteins have been shown to be arranged in a rod-like conformation and are depicted in Fig. 2A as such (Engel, J. (1989). EGF-like domains in extracellular matrix proteins: localized signals for growth and differentiation. FEBS. 251, 1-7) with the individual EGF

**Fig. 3. Comparison of in situ, Antibody and Enhancer Trap Staining**

The SLIT message, protein, and promoter activation are visualized at three stages of embryogenesis by in situ hybridization (A, D, G and J), antibody staining (B, E, H and K) and enhancer trap detection (C, F, I and L). The following stages during embryogenesis are shown: gastrulation in a dorsal view (A, B and C), germ band extended stage in a dorsal view (D, E and F) and nerve cord condensation, from both dorsal (G, H and I) and sagittal views (J, K and L). Staining can be demonstrated by all three methods in the midline neuroepithelium (arrow in D, E, F), midline glial cells (bold arrow in G, H, I, J, K) and cardioblast (open arrow in J, K, L), as well as in the walls of the gut and in a segmentally-reiterated pattern near the muscle attachment sites (thin arrow G, H, I). While no signal above background is detected from the lateral neuronal cell bodies, antibody staining (long thin arrow in H) is visible on the axonal projections from these neurons.

**Fig. 4. Confocal Localization of the SLIT Protein to Cardioblasts and Muscle Attachment Sites**

Fig. 4A depicts an optical, horizontal section of an embryo undergoing dorsal closure stained with anti-SLIT antibodies shows the SLIT protein to be localized on the surface of cardioblasts (opposing arrows) and at the muscle attachment sites to the body wall (long arrow).
Fig. 4B depicts a higher magnification view of the cardioblasts and shows that the highest concentration of the SLIT protein is localized to the regions of contact (long arrow) between opposing pairs of cardioblasts (apposing arrows) as they come together to form the lumen of the larval heart.

Fig. 4C is a sagittal view (dorsal side up) that shows the SLIT protein to be localized to the sites of muscle attached to the ectoderm (long arrows). Autofluorescence from the gut is also visible.

Fig. 5. Immunoelectron Microscopic Localization of SLIT in the Embryonic CNS to Midline cells and Axonal Tracts

Staining with anti-SLIT antibody in a frontal section through the plane of the longitudinal and commissural axonal tracts, detected by silver intensification of an HRP-conjugated secondary antibody. At the E.M. level labeling is both on the axons comprising the longitudinal connectives (lc), anterior (ac) and posterior (pc) commissures and on the cells lying between them including the processes of the midline glial cells (arrows). A light level frontal view of a similarly prepared dissected nerve cord shows strong axonal labeling with respect to the midline cells (see insert). No signal above background is seen on lateral neuronal cell bodies (N) either at the light or electron microscopic level. (scale bar = 5μm.)

Fig. 6. Secretion of SLIT from Cultured Cells

Fig. 6A depicts an immunoblot with anti-SLIT antibodies of the SLIT protein immunoprecipitated from embryos (Lane 1) and S2 culture cells Lane 2), shows a common protein species of approximately 200kD (arrow). This species is also
immunoprecipitated from S2 cell line conditioned media (Lane 3) indicating that the SLIT protein can be exported from the cells in which it is produced. Lane 4 shows by immunoblotting that the 200kD SLIT protein species can also be detected in the matrix materials deposited by the S2 cells in culture. Predominant band seen in immunoprecipitations is immunoglobulin heavy chain (indicated by an H).

In Fig. 6B the media in which S^35 metabolically-labeled S2 cells had been cultured was immunoprecipitated with anti-SLIT antibodies, separated by SDS-page, and detected by autoradiography. Consistent with the immunoblotting results, a major 200 kD species is detected (arrow). Tick marks indicate position of 100 kD and 220 kD molecular weight size standards.

**Fig. 7. Null Mutant Embryos Exhibit Disruptions in Midline Cells**

The pattern of expression of β-galactosidase in the MP2 cells (A,B) and the midline neuroepithelium and its progeny (C-H) is compared in wild type and null mutant embryos. Anterior is toward the left.

(A,B): A dorsal view shows the MP2 cells (arrows) well separated by cells of the midline neuroepithelium at the extended germband stage in wild-type embryos (A) but closer together in a SLIT mutant background (B), indicating an early disruption along the midline.

(C,D): The midline neuroepithelium at the germband extended stage (arrow in C) and its midline progeny (E,G) are clearly labeled in wild type embryos. In comparison, following germband extension in slit mutant embryos there is either no midline neuroepithelial labeling, or low levels of labeling slightly later (arrow in D).

(E,F): A sagittal view during nerve cord condensation shows the bulk of the midline cells of each neuromere clearly expressing
β-galactosidase in the wild-type embryo (arrow in E). However, in slit mutant embryos, the expressing cells are reduced in number and displaced to the ventral edge of the nerve cord (arrow in F).

(G,H): A dorsal view of a similarly staged wild type (G) and SLIT mutant (H) embryo. In the wild type the midline cells can be seen in the space separating adjacent neuromeres within a segment. In SLIT mutant embryos, expressing cells can be seen to lie irregularly shifted laterally as well as ventrally (arrow).

Fig. 8. Levels of SLIT Expression Correlate with Disruptions of Midline Cells and Axon Pathways

The major axonal pathways are labeled with anti-HRP antibodies (A,C,E) (Jan, L. Y., and Jan, Y. N. (1982). Antibodies to horseradish peroxidase as specific neuronal markers in Drosophila and grasshopper embryos. Proc. Natl. Acad. Sci. USA 79, 2700-2704) and compared to the staining pattern seen with antibodies against the SLIT protein (B,D,F). In these horizontal views anterior is toward the left.

(A,B): In wild type embryos the ladder-like arrangement formed by the commissural and longitudinal axonal tracts is visible. Staining with antibodies against the SLIT protein (B) shows labeling of the midline glial cells (thick, mid-sized arrow) as well as axonal staining (short arrow).

(C,D): Anti-HRP stained null mutant embryos (C) exhibit a single centrally located longitudinal nerve bundle along the length the CNS. No detectable SLIT staining is seen (D). The lateral neuronal bodies are shifted inward toward the center, filling the space normally occupied by the midline cells. An overall reduction in the width of the nerve cord is also observed (double-ended arrow).
(E,F): slit^{E138} mutants exhibit an intermediate phenotype characterized by a partial collapse of the axonal scaffold. Relatively weak SLIT staining is visible along the length of the axonal bundles (F). Segments with the highest levels of SLIT staining (arrow), have more midline cells and a less severe collapse of the longitudinal connectives (short arrow) in comparison to segments with lower expression levels (long arrow). Segments with reduced levels of slit expression exhibit nervecord compression and a concomitant fusion of the axon tracts (long arrow).

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ. I.D. NO. 1

The SLIT Nucleotide Sequence Codes for a Putative Extracellular Protein with Both Flank-LRR-Flank and EGF domains

The cDNA sequence containing the slit coding region is shown as SEQ. I.D. NO. 1. The coding domain is characterized by the presence of a putative signal sequence and four distinct blocks of leucine-rich repeats followed by two regions containing epidermal growth factor repeats. The location of the predicted signal sequence cleavage site is indicated. There are 13 potential N-linked glycosylation and two consensus sequences for β-hydroxylation (Rees, D. J. G., Jones I. M., Handford, P.A., Walter, S. J., Esnouf, M. P., Smith, K. J., and Brownlee, G. G. (1988)). The role of β-hydroxyaspartate and adjacent carboxylate residues in the first EGF domain of human factor IX. EMBO J. 7,2053-2061) in the third and fifth EGF repeats. The 33bp alternatively spliced segment in the slit transcript, and the 11 amino acids which it encodes are shown.
SEQ. I.D. NO. 2
Amino acid sequence of the entire SLIT protein, including four Amino-flank-LRR-Carboxy-flank domains, 6 tandem EGF-like repeats, an intervening region, the 7th EGF-like repeat, an alternative splice segment, and a carboxy terminal region.

SEQ. I.D. NO. 3
Amino acid sequence of the first Amino-flank-LRR-Carboxy-flank domain of SLIT protein.

SEQ. I.D. NO. 4
Amino acid sequence of the second Amino-flank-LRR-Carboxy-flank domain of SLIT protein.

SEQ. I.D. NO. 5
Amino acid sequence of the third Amino-flank-LRR-Carboxy-flank domain of SLIT protein.

SEQ. I.D. NO. 6
Amino acid sequence of the fourth Amino-flank-LRR-Carboxy-flank domain of SLIT protein.

SEQ. I.D. NO. 7
Eleven amino acid alternative splice segment.

SEQ. I.D. NO. 8
Concensus amino acid sequence for an Amino-flank-LRR-Carboxy-flank domain.

SEQ. I.D. NO. 9
Carboxy-terminal region of the SLIT protein.
DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns the full structure of the SLIT protein, sequence elements thereof and the design of diagnostic and therapeutic reagents based on the elucidation of their role in biological systems.


In addition, it is demonstrated herein that sequences flanking the LRRs of SLIT exhibit homology to sequences in corresponding positions in some of the other LRR-containing proteins. It is also demonstrated herein that SLIT is necessary for the normal development of the midline of the CNS, including in particular the midline glial cells, and for the concomitant formation of the commissural axon pathways. Furthermore, this process is dependent on the level of SLIT protein expression. Data is provided herein indicating that the SLIT protein is excreted from the midline glial cells where it is synthesized, and is eventually associated with the surfaces of the axons that traverse them. In addition, the SLIT protein is tightly localized to the muscle attachment sites and to the sites of contact between adjacent pairs of cardioblasts as they coalesce to form the lumen of the larval heart. The implications of the structure and distribution of the SLIT protein in development are discussed in detail hereinbelow.

Molecular Characterization of the SLIT Transcript and P-element Alleles

The isolation and partial characterization of SLIT EGF-homologous sequences and corresponding cDNA clones was
described previously (Rothberg et al. 1988 supra). Applicants have extended this molecular analysis to include the entire SLIT coding sequence, its genomic organization, characterization of a splicing variant, and the molecular basis of four P-element induced mutations. The SLIT embryonic transcript was estimated to be approximately 9kb by Northern analysis. Using both conventional hybridization screening procedures and methods employing the polymerase chain reaction (PCR), applicants obtained cDNA clones representing 8.6kb of this sequence. Sequencing of genomic DNA indicates a consensus Drosophila transcriptional initiation sequence (Hultmark, D., Klemenz, R., and Gehring, W. J. (1986). Translational and transcriptional control elements in the untranslated leader of the heat-shock gene hsp22. Cell 44,429-438, 1986) 53bp upstream of applicants' longest cDNA.

Fig. 1 shows the SLIT transcript aligned with a restriction map of the corresponding genomic regions. The known intron/exon boundaries are indicated in Fig. 1A and were determined by a comparison of the cDNA sequence with known genomic sequence (Rothberg et al., 1988 supra). The SLIT cDNA sequence spans an approximately 20kb genomic region and contains a single 4440 bp open reading frame (ORF). The nucleotide and deduced amino acid sequences of the ORF are shown in SEQ. I.D. NO. 1.

Restriction mapping and sequence analysis of SLIT cDNA clones revealed two classes of transcript differing by 33 nucleotides. The location of this sequence variation is shown in SEQ. I.D. NO. 1. The presence of a minor sequence variation prompted a more careful analysis of slit cDNA clones in order to detect whether other transcript variants existed that might not have been detected by Northern analysis. Utilizing a cDNA screening procedure based on the PCR, the only detectable size variation was confined to the same region as in the original variant. A comparison of the genomic and cDNA sequences demonstrates that the 33 nucleotide size variation is the result of alternate RNA splicing. The two species of SLIT cDNA differ in the location of a donor (5') splice site, while the acceptor (3') site is identical.

The molecular characterization was been extended to include the determination of the site of P-element insertion.
in four SLIT alleles slit^{F81}, slit^{f119}, slit^{E158} and slit^{175}, which were recovered during a P-element based enhancer trap screen (Bier E, et al., (1989)). Searching for pattern and mutation in the Drosophila genome with a P-lacZ vector. Genes & Dev. 3, 1273-1287; Bellen, H. J., O'Kane C. J. Wilson, C., Grossniklaus, U., Pearson, R. K. and Gehring, W. Y. (1989) P-element-mediated enhancer detection: a versatile method to study development in Drosophilia, Gen. & Dev. 3, 1273-1287). Genomic DNA from each line was employed in the PCR using primers designed to detect P-element insertions in regions 5' of the SLIT coding sequence. By direct sequencing of the PCR products, these lines were shown to contain insertions upstream of both the SLIT consensus transcription initiation sequence and ORF (see Fig. 1B) confirming their initial characterization as SLIT alleles and suggesting their utility in the characterization of SLIT expression.

SLIT Codes for Flank-LRR-Flank and EGF Domains

The SLIT transcripts potentially encode two proteins of 1469 and 1480 amino acids, with molecular weights of approximately 166kD. The predicted initiating methionine is followed by an amino acid sequence containing structural regions characteristic of a secretory signal sequence (SEQ I.D. NO. 1). However, hydropathy plots do not predict a transmembrane domain (data not shown). An examination of the
SLIT coding domain reveals that the majority of the protein is composed of two repeated motifs: the 24 amino acid leucine-rich repeat (LRR) and the 40 amino acid EGF repeat (SEQ. I.D. NO. 1). Fig. 2A shows schematically the positions of these repeats and indicates a higher level of organization among the LRRs. The LRRs are arranged in four groups, each composed of four or five LRRs surrounded by conserved amino- and carboxy-flanking regions (Fig. 2B) (SEQ. I.D. NOS. 3, 4, 5 and 6). The presence of both the LRRs and EGF-like repeats within a single protein make SLIT unusual; this combination is not found in any other proteins in the NBRF databank. The absence of any potential transmembrane domains in a sequence having a typical signal sequence and two known extracellular-associated motifs suggests that the SLIT locus encodes a secreted extracellular protein.

The LRR motif is found in a variety of vertebrate and invertebrate proteins involved in protein-protein interactions (Table 1).
Table 1. Leucine-rich Repeat Containing Proteins

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Arrangement</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoprotein lbz</td>
<td>LRR-Flank</td>
<td>Receptor/Adhesion</td>
<td>Titanl et al., 1987; Lopez et al., 1987</td>
</tr>
<tr>
<td>Glycoprotein lbB</td>
<td>Flank-LRR-Flank</td>
<td>Receptor/Adhesion</td>
<td>Lopez et al., 1988</td>
</tr>
<tr>
<td>Glycoprotein IX</td>
<td>Flank-LRR-Flank</td>
<td>Receptor/Adhesion</td>
<td>Hickey et al., 1989</td>
</tr>
<tr>
<td>Lutropin-Chorlogonadotropln receptor</td>
<td>LRR</td>
<td>Receptor</td>
<td>McFarland et al., 1989</td>
</tr>
<tr>
<td>Collagen-binding 59 kd protein (fibromodulin)</td>
<td>Flank-LRR</td>
<td>ECM binding</td>
<td>Oldberg et al., 1989</td>
</tr>
<tr>
<td>Small Intestinal proteoglycan PG-S1 (Biglycan)</td>
<td>Flank-LRR</td>
<td>ECM binding</td>
<td>Fisher et al., 1989</td>
</tr>
<tr>
<td>Small Intestinal proteoglycan PG-S2 (Decorin, PG-40)</td>
<td>Flank-LRR</td>
<td>ECM binding</td>
<td>Krusius et al., 1986; Day et al., 1987</td>
</tr>
<tr>
<td>Adenylylate cyclasea</td>
<td>LRR</td>
<td>Protein-Protein</td>
<td>Kataoka et al., 1985; Feld et al., 1990</td>
</tr>
<tr>
<td>Ribonuclease/angiogenin inhibitora</td>
<td>LRR</td>
<td>Protein-Protein</td>
<td>Schneider et al., 1988</td>
</tr>
<tr>
<td>Chaoptin</td>
<td>LRR</td>
<td>Homotypic Adhesion</td>
<td>Reinke et al., 1988; Krantz et al., 1990</td>
</tr>
<tr>
<td>Leucine-rich α2 glycoprotein</td>
<td>LRR</td>
<td>??</td>
<td>Takahashi et al., 1985</td>
</tr>
<tr>
<td>Oligodendrocyte-myelin Glycoprotein</td>
<td>Flank-LRR</td>
<td>Adhesion?</td>
<td>Mikol et al., 1990</td>
</tr>
<tr>
<td>Toll</td>
<td>2x</td>
<td>LRR-Flank</td>
<td>Dorsal-ventral polarityb</td>
</tr>
<tr>
<td>sH</td>
<td>4x</td>
<td>Flank-LRR-Flank</td>
<td>Morphogenesis b</td>
</tr>
</tbody>
</table>

a Intracellular proteins, all other are extracellular or cell surface proteins.

b While the role of these proteins in Drosophila development is known, it is not known how their function is mediated.
References Listed in Table 1


Fisher, L. W., Termine, J. D., and Young, M. F. (1989). Deduced protein sequence of bone small proteoglycan (Biglycan) shows homology with proteoglycan II (Decorin) and several nonconnective tissue proteins in a variety of species. J. Biol. Chem. 264, 4571-4576.


Together with their surrounding sequences, the tandem arrays of LRRs in SLIT form a Flank-LRR-Flank structure, part of which was previously noted in some of the other LRR-containing proteins (Hickey et al., 1989 supra). However, in this application, applicants extend both the amino-terminal LRR flanking sequence and the carboxy-terminal flanking sequences to include invariant cysteines, arginines, prolines, and other conserved residues (consensus in SEQ. I.D. NO. 8). A comparison of other LRR-containing proteins with SLIT reveals that a subset have homology to SLIT extending to either one or both of the conserved flanking regions as defined herein (Table 1; SEQ. I.D. NO. 8). This similarity is found in the oligodendrocyte-myelin glycoprotein (OMgp) of humans, the Toll gene of Drosophila melanogaster and among two sets of structurally related vertebrate proteins involved in adhesive events. OMgp is believed to mediate the adhesion of oligodendrocytes to either other glial cells or axons (Mikol, et al., 1990 supra) and contains the amino-flanking region and 7 LRRs. Toll, a transmembrane protein, is required for dorsal-ventral pattern formation (Hashimoto, C., Hudson, K. L., and Anderson, K.V. (1988). The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell 52,269-279) and has an
extracellular domain characterized by the presence of two LRR regions with SLIT homologous carboxy-flanking sequences.

The first set of vertebrate proteins with slit homology in their flanking regions comprise the von Willebrand factor receptor (Titani et al., 1987, supra; Lopez et al., 1988, supra; Hickey et al., 1989, supra). The similarities between SLIT and two members of this protein complex, GPIX and GPIbβ, include the full Flank-LRR-Flank motif, albeit with a single LRR. The third member of this complex GPIbα, however, contains a tandem array of LRRs and a conserved carboxy-flanking region without a conserved amino-flanking region. Extensive similarity between SLIT and a second group of vertebrate proteins is apparent in their LRR and amino-flanking regions. This group consists of the ECM proteoglycans decorin (Day, A. A., McQuillan, C. I., Termine, J. D., Young, M. R. (1987). Molecular cloning and sequence analysis of the cDNA for small proteoglycan II of bovine bone. Biochem. J. 248, 801-805; Krusius and Ruoslahti, 1986, supra) and biglycan (Fisher et al., 1989, supra) and fibromodulin (Oldberg, 1989, supra). These proteins have overall homology to one another and define a family of extracellular proteins with conserved amino-flanking regions and 10 consecutive LRRs (Oldberg et al., 1989, supra).

All the proteins exhibiting homology to SLIT in their LRR flanking regions have either been shown, or are believed, to participate in extracellular protein-protein interactions. Moreover, SLIT contains 7 copies of the EGF motif (Fig. 2A), which also has been shown to participate in extracellular protein-protein interactions (Rothberg et al., 1988 supra). The last EGF repeat is of special interest because the alternate mRNA splicing noted earlier potentially results in the insertion or removal of 11 unique amino acids at the COOH terminal of this repeat (see SEQ. I.D. NO. 7) (Fig. 2A).
SLIT is Exported From Glial Cells and Distributed Along Axon Tracts

It has been shown previously that SLIT transcript and protein could be detected at the highest levels in the midline glial cells (Rothberg et al., 1988 supra). However, despite the presence of the SLIT protein on the axons in the embryonic commissural and longitudinal axon pathways, applicants failed to detect any transcript or protein in the cell bodies of these neurons. This raised the possibility that the SLIT protein, which is synthesized in and presumably secreted by the midline cells, can become associated with axons. Here this question is further explored in whole-mount embryo preparations by comparing the sites of SLIT expression, as assayed by in situ hybridization and the detection of β-galactosidase in SLIT enhancer trap lines, with the subsequent localization of the protein as assayed by antibody staining (summarized in Fig. 3).

All four enhancer trap alleles (slit$^{f81}$, slit$^{f119}$, slit$^{f158}$ and slit$^{175}$) express β-galactosidase within the ventral midline to varying levels. The location of the P-element constructs 5' of the SLIT coding domain, the resulting mutant phenotypes and especially their expression patterns are all consistent with their being under the transcriptional control of SLIT regulatory elements. A summary of the embryonic localization of the SLIT mRNA and protein, and the β-galactosidase expression of slit$^{f158}$ is shown in Fig. 3. The expression of β-galactosidase from the enhancer trap construct in slit$^{f158}$ shows excellent overall agreement with mRNA localization data at all embryonic stages (compare Figs. 3A, D, G and J with 3C, F, I and L). Each method reveals a nearly identical expression pattern starting at gastrulation (Fig. 3A, B and C). At germ band extension, all of the midline mesectodermal cells (Crews, S.T., Thomas, J. B., and Goodman, C. S. (1988)
The Drosophila single-minded gene encodes a nuclear protein with sequence similarity to the per gene product. Cell 52, 143-151; Thomas, J. B., Crews, S. T. and Goodman, C. S. (1988). Molecular genetics of the single-minded locus: a gene involved in the development of the Drosophila nervous system. Cell 52, 133-141) show the highest level of slit expression (Figs. 3D, E and F). During germband retraction and nerve cord shortening, expression is most restricted to the six midline glial cells which are derivatives of the midline neuroepithelium (Figs. 3G, H, and I). Localized expression is also evident in the cardioblasts (Figs. 3J, K and L) during dorsal closure. Figs. 4A and B show that the SLIT protein is most highly localized to the points of contact between opposing pairs of cardioblasts as they coalesce to form the dorsal vessel (presumptive larval heart). All three methods also reveal expression in the walls of the gut (Figs. 3J, K and L) and in a segmentally-reiterated pattern near the muscle attachment sites in the ectoderm (apodemes; Figs. 3G, H and I). Precise protein localization to the sites where the muscles are attached to the apodemes is seen by confocal microscopy (Figs. 4A and C).

In situ hybridization (Figs. 3D, G and J) and the expression from the enhancer trap lines (Figs. 3F, I and L) both support the observation that initially all of the midline cells, and subsequently primarily the six midline glia, are producing SLIT while lateral neurons are not. However, antibody labeling is seen strongly in the midline glia (Fig. 3E, H) and on the commissural and longitudinal axon tracts (Fig. 3E, H and K), while it is absent from lateral neuronal cell bodies, which supply the bulk of the axons to these bundles. These results suggest that the antibody labeling along the commissural and longitudinal axon tracts is due to the distribution of SLIT protein exported from the midline
glial cells. The protein is also absent from the peripheral nerve roots and peripheral axon tracts.

Immunoelectron microscopy was used to determine the subcellular localization of the SLIT protein in the ventral nerve cord. Dissected embryonic nerve cords demonstrate staining on the midline cells as well as on the commissural and longitudinal nerve bundles. Light and electron micrographs of a similarly prepared sample are shown in Fig. 5. While all the derivatives of the neuroepithelium initially express SLIT, during nerve cord condensation and axonal outgrowth this expression becomes restricted to the midline glial cells. The midline glial cells surround the developing commissural axons and growth cones have been shown to track along their surface (see Jacobs and Goodman, 1989, J. Neurosci., 9, 2402-2411). Antibody staining can be seen both on the surface of the midline glial cells where they abut growing axons and on the axons themselves. No detectable variation in the amount of SLIT staining among subsets of axons or fascicles is detected.

Applicants were able to detect SLIT along the length of the axonal projections in the commissural and longitudinal axon tracts though we are unable to detect any signal above background from the lateral neuronal cell bodies supplying these axonal (Fig. 5). Immunoelectron microscopy demonstrated the extracellular localization of the SLIT protein and supports the expression data indicating that the SLIT protein on the axon tracts is not produced by the neurons whose axons comprise them. Thus, it appears that the axonally distributed SLIT protein is first secreted from the midline glial cells and then becomes associated with these axons as they traverse the midline.
To obtain direct biochemical evidence that SLIT is exported from the cells in which it is produced, applicants investigated SLIT expression in Drosophila tissue culture cell lines. Schneider line S2 was found to normally express the SLIT protein, and it can be seen on the surface of a subset of the cells by immunofluorescence. Immunoblotting of immunoprecipitated protein extracts from Drosophila embryos and S2 cell lines revealed a single 200kD molecular weight band (Fig. 6A, lanes 1 & 2). This size is consistent with expectations of a glycosylated form of the predicted SLIT protein. Conditioned Schneider cell media also was found to contain a similar 200kD molecular weight species (Fig. 6A, lane 3) in addition to two other species which may represent differences in glycosylation. The presence of the SLIT protein in the culture media was confirmed by immunoprecipitations of the same molecular weight species from media in which $^{35}$S metabolically-labeled S2 cells had been growing (Fig. 6B). These experiments further support the suggestion that SLIT is an excreted protein. Additionally, immunoblotting of the matrix materials deposited in culture by S2 cells showed the SLIT protein to be enriched in this fraction (Fig. 6A, lane 4), consistent with the hypothesis that SLIT functions as an extracellular matrix molecule.

SLIT Mutants Exhibit Disruptions in Midline Cells and Commissural Axon Pathways

An analysis of SLIT null mutant embryos reveals the collapse of the normal scaffold of commissural and longitudinal axons. However, the SLIT protein is detectable in the midline neuroepithelial cells well before the time of axonal outgrowth (Rothberg, 1988 supra). This raised the possibility that the SLIT protein influences the differentiation of midline cells from the neuroepithelium and that the observed collapse of the axonal scaffold is the
result of an earlier developmental abnormality. In order to
examine the development of the midline before axon outgrowth,
applicants followed the fate of the MP2 cells (an identified
neuronal precursor cell that normally develops in the most
medial row of neuroblasts in the lateral neuroepithelium) as
well as the midline neuroepithelium and its progeny in both
wild-type and mutant embryos.

In wild-type embryos at the germband-extended stage the
MP2 cells are separated by the midline neuroepithelium (Fig.
7A), whereas in SLIT embryos these cells appear closer
together (Fig. 7B). In addition, cell autonomous markers
(lines 8-7 & 242) for some of the midline neuroepithelial
cells and their progeny (Fig. 7C, E, G) are either absent or
ectopically expressed before (Fig. 7D) and during axonal
outgrowth (Fig. 7F, H). For example, in SLIT mutant embryos,
some of these cells appear absent and others come to lie in an
abnormal position along the ventral surface of the nerve cord
(Fig. 7F, H). These results clearly show a perturbation in
the development of the midline neuroepithelial cells as early
as the extended-germband stage. This disruption further leads
to a disruption of their progeny, including the midline glial
cells, resulting in a lateral compression of the nerve cord
(confirmed by histological analysis). Given the disruption in
the development of the midline of the CNS, the ensuing
collapse of the axonal scaffold is not unexpected (a similar
phenotype of the stimulant; Crews et al., 1988, supra; Thomas
et al., 1988, supra).

Mutations caused by the insertion of the enhancer-trap P-
element allow for a further exploration of the relationship
between the level of SLIT expression and the extent of the
nerve cord defect. In the wild-type embryo, as observed with
antibodies specific to neuronal membranes, commissural and
longitudinal axon pathways appear to form a regular ladder-
like structure (Fig. 8A). A wild-type embryo stained with anti-SLIT antibodies also shows labeling of the CNS axon pathways, as well as prominent staining of the midline glial cells (Fig. 8B). Embryos homozygous for slit$^{1607}$ do not have any detectable SLIT expression either in the midline cells or on the axonal bundles (Fig. 8D). Thus null allele is embryonic lethal; mutant embryos exhibit a lateral compression of the nerve cord (Fig. 8D), and a single fused longitudinal axon tract (Figure 8C).

As judged by antibody staining intensity in whole-mount embryo preparations, all four enhancer trap SLIT alleles show reduced levels of SLIT expression in the homozygous state at 18°C and exhibit an intermediate phenotype. Since the P-element construct resides upstream of SLIT coding sequences, it is reasonable to assume that it is not the disruption of the SLIT protein per se that is responsible for the observed mutant phenotypes, but rather a reduction in the level of SLIT expression. These mutations are embryonic and larval lethals and in contrast to the null allele slit$^{1607}$, exhibit only partial compression of the midline and a concomitant partial collapse of the axonal scaffold (Fig. 8E and F). Variable levels of SLIT expression in the midline cells, often at lower levels and in a more diffuse pattern were noted compared to wild type. This variability is seen both between individual embryos and between segments in the same embryo (Fig. 8F). The segments with the lowest levels of expression exhibit the least differentiation their midline cells, including their midline glia, and show the greatest degree of collapse of both the ventral nerve cord and the axon tracts (Fig. 8F). Segments exhibiting higher levels of expression appear at a gross level to have nearly normal midline glial cells, commissures, and longitudinal axon tracts (Fig. 8F).
As mentioned herein, it is demonstrated herein that the SLIT locus, whose mutant phenotypes indicate that it plays a major role in the development of the specialized midline glial cells and the commissural axon tracts that traverse them, encodes a unique extracellular protein containing two structural motifs associated with adhesive interactions. The SLIT protein has four regions containing tandem arrays of a 24 amino-acid leucine-rich repeat (LRR) with conserved flanking sequences (Flank-LRR-Flank) and two regions with epidermal growth factor (EGF)-like repeats. Although the LRR and EGF motifs are not found together in any other proteins in the NBRF data bank, each has been found in conjunction with other sequence motifs, often forming a distinct region of a larger protein involved in protein-protein interactions. As part of larger proteins, each of these motifs has been shown to directly contribute to these interactions.

The LRRs in SLIT are similar to those that were first identified in human leucine-rich α2-glycoprotein and later in a variety of vertebrate and invertebrate proteins involved in protein-protein interactions, both inside and outside the cell (Table 1). In the extracellular environment, the LRRs have been found in conjunction with a variety of conserved protein motifs (McFarland et al., 1989 supra; Mikol et al., 1990 supra). Of greatest interest, however, is the fact that the LRRs in extracellular proteins are often found accompanied by either one or both of the conserved amino- and carboxy-flanking regions identified in the slit protein (see Table 1). In all of the cases where the LRR are accompanied by these flanking regions the proteins have either been shown, or are believed, to participate in extracellular adhesive interactions. While the significance of the individual flanking regions in these interactions is not yet known, a functional role for at least the carboxyl-flanking sequence has been demonstrated in vivo: mutations in the cysteines of
this region in the Drosophila Toll protein confer a dominant phenotype.

In addition to Toll and the oligodendrocyte-Myelin glycoprotein, two distinct families of adhesive proteins have SLIT homology extending to the LRR flanking sequences. The first includes a set of functionally related interstitial proteoglycans known to bind directly to ECM proteins: biglycan, fibromodulin and decorin. Biglycan binds laminin and fibronectin, while fibromodulin and decorin bind collagen and fibronectin and have a regulatory effect on collagen fibril formation (Vogel K. G., Paulsson M., and Heinegard, D. (1984). Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. Biochem. J. 223, 587-597; Hedbom, E., and Heinegard, D. (1989). Interactions of 59-kDa connective tissue matrix protein with collagen I and collagen II. J. Biol. Chem. 264, 6898-6905; Oldberg et al (1989) supra; Schmidt, G., Robeneck. H., Harrach, B., Glossl, J., Nolte, V., Hormann, H., Richter, H., and Kresse, H. (1987). Interactions of small dermatan sulfate proteoglycan from fibroblasts with fibronectin. J. Cel. Biol. 104, 1683-1691). The second set comprises the proteins of the glycoprotein Ib-IX (GPIb-IX) complex, which together function as a receptor for the von Willebrand factor (vWF) and thrombin and are responsible for vWF-dependent platelet to blood vessel adhesion. In this complex, the LRR-containing region of the GPIbα chain binds one of a set of three repeated 200 amino acid sequences termed A domains in vWF (Titani et al, 1987 supra; Mohri H., (1988) Structure of the von Willebrand Factor Domain Interacting with Glycoprotein Ib. J. Biol. Chem., 17901-17904). In addition to demonstrating the role of the LRR motif in protein-protein interactions this homology also raises the possibility that similar regions in SLIT might bind to proteins containing repeats homologous to the A domains of vWF. In vertebrates, these proteins include both ECM

The conservation of the amino-terminal sequences flanking a LRR region in a family of proteins that participate in direct adhesion to ECM components suggests that this structure may play a similar role in SLIT. Alternatively the conversation of the entire Flank-LRR-Flank motif in SLIT and the GPlb-IX complex offers the intriguing possibility that SLIT's interactions with the ECM, like those of the vWF and thrombin receptor, could be mediated by additional factors.

In comparing the various proteins known to contain the EGF-like motif, it is clear that this sequence is always found in an extracellular environment and in many instances these sequences have either been implicated, or shown, to function directly in protein-protein interactions. (Apella, E., weber, I.T., and Blasi, F. (1988). Structure and function of epidermal growth factor-like regions in proteins. FEBS. 231, 1-4).

In addition, these repeats are found in conjunction with a variety of other structural and catalytic domains in molecules involved in blood coagulation (Furie, B., and Furie, B. C. (1988). The molecular basis of blood coagulation. Cell 53, 505-518) and in adhesive ECM glycoproteins (Engel, 1989, FEBS, 251, 1-7)). Tandem arrays of EGF-like repeats comprise the majority of the extracellular domains of the cell surface proteins Notch (Wharton, K. A., Johansen, K. M., Xu, T., and Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus Notch implies a gene product that shares homology with proteins containing EGF-like repeats.
Cell 43, 567-581) and Delta (Vassin, H., Bremer, K. A., Knust, E., and Campos-Ortega, J. (1987). The neurogenic gene Delta of Drosophila Melanogaster is expressed in neurogenic territories and encodes and putative transmembrane protein with EGF-like repeats. EMBO J. 6, 3431-3440). 1987; Kopczynski et al., 1988) and have been implicated in Ca++ dependent heterotypic adhesive interactions between the two proteins as well as in homotypic interactions in the Delta protein (Fehon et al., 1990, Cell, 61, 523-534).

The EGF-like repeats in SLIT are arranged in two groups in a fashion similar to the arrangement found in cell surface and extracellular adhesive proteins and in EGF-like ligands, respectively (Apella et al., 1988 supra; Lander, A. D. (1989). Understanding the molecules of neural cell contacts: emerging patterns of structure and function. TINS. 12, 189-195). An additional similarity between the EGF-like repeats in SLIT, Delta and Notch is a conserved recognition site for a post-translational modification involved in Ca++ binding (Rees et al., 1988, EMBO J. 2053-2061) and a consensus sequence implication in Ca++ dependent protein-protein interactions (Handford, P.A., Baron, M., Mayhew, M., Willis, A., Beesley, T., Brownlee, G. G., and Campbell, I.D. (1990). The first EGF-like domain from human factor IX contains a high-affinity calcium binding site. EMBO J. 9, 475-480).

By these criteria the 3rd and 5th EGF-repeats of SLIT are potential candidates for β-hydroxylation and may participate in Ca++ dependent interactions. The 7th and last EGF domain in SLIT is separated from the tandemly arranged EGF-repeats by 202 amino acids.

Export and Cell Binding

Using both whole-mount in situ hybridization and SLIT enhancer trap alleles, applicants were able to demonstrate
that SLIT is produced in the developing midline neuroepithelium, as well as in its progeny midline glial cells along the dorsal midline of the CNS, but not in the neuronal cell bodies whose axons form the major commissural and longitudinal axon tracts in the CNS. Light and immunoelectron microscopy indicate that SLIT is exported from the midline glial cells and is associated with the axons that traverse them. If, as is suggested by this data, the SLIT gene product is not produced in the neurons of the axons on which it resides, it is expected that it is secreted from the midline cells and "picked up" by passing axons. This in turn raises the possibility that the axons that carry SLIT on their surface may be expressing specific receptors capable of interacting with SLIT in a direct or indirect manner. An analysis of SLIT expressing in Drosophila cell culture demonstrates that SLIT can in fact be localized to the surface of individual cells. Additional biochemical support for the extracellular, secreted nature of the protein was provided by demonstrating that tissue culture cells producing SLIT are secreting the protein into the media. Moreover, consistent with the hypothesis that SLIT functions as an ECM molecule, it was found that the protein to be accumulated in the matrix materials deposited by these cells.

Morphogenetic Regulation of the Neuroepithelium

A model for SLIT function wherein it regulates the morphological differentiation of a cell by attaching to both the ECM and cell surface receptors is consistent with its predicted structure, its expression pattern and phenotype. Like the other ECM glycoproteins, SLIT is composed of repetitive structural motifs and lacks the hydrophilic regions characteristic of membrane-spanning cell-surface adhesion molecules. ECM glycoproteins play a diverse role in development, acting as signals for cell differentiation,
growth and migration. Furthermore, the SLIT-homologous proteoglycan decorin is involved in the control of cell proliferation and has the ability to convert transformed cells to morphological regularity (Yamaguchi, Y., and Ruoslahti, E. (1988). Expression of human proteoglycan in Chinese hamster ovary cells inhibits cell proliferation. Nature 336, 244-246).

SLIT's involvement in the development and differentiation of the midline neuroepithelium and the subsequent formation of commissural axon pathways is demonstrated herein. In a SLIT mutant background the midline cells do not undergo proper differentiation or morphological movements; instead of filling the midline of each neuromere as they do in the wild type embryo, they appear at the base of the nerve cord and are fewer in number. This is followed by the complete collapse of the axonal scaffold. The in vivo effects of reductions in SLIT expression further indicate that the morphogenesis of the midline cells and the subsequent axonal pathway formation are dependent on the concentration of slit protein. Using P-element induced SLIT alleles, applicants were able to demonstrate that a reduction in SLIT expression is coincident with the lack of development of an individual segment's midline cells, and specifically, with the development of the midline glial cells. It was further demonstrated that the variability in the extent of collapse of the midline of the nerve cord is mirrored by the extent of collapse of the commissural and longitudinal axon pathways.

It is noted with interest that the extent of disruption in the ventral nerve cord in slit alleles corresponds to the range of phenotypes exhibited by mutations of the Drosophila EGF-receptor homolog (DER). Given the homology between SLIT and EGF-receptor ligands, the co-localization of the DER and SLIT proteins to the midline glial cells and the muscle
attachment sites (Zak. N. B., Wides, R. J., Schejter, E.D., Raz, E., and Shilo, B. (1990). Localization of the DER/flb protein in embryos: implications on the fit little bal lethal phenotype. Development, 109, 865-874) raises the possibility that SLIT functions as a DER ligand. This speculation is particularly attractive since the activation of a receptor tyrosine kinase by the SLIT protein would offer a mechanistic explanation for SLIT's influence on either the development or maintenance of the midline cells and provide for a direct molecular link between the ECM and genes involved in cellular proliferation and differentiation (Yarden, Y., and Ullrich A. (1988) Growth factor receptor tyrosine kinases. Ann. Rev. Biochem. 57, 443-78).

Implications of SLIT Expression

The three major regions of SLIT expression are the (1) midline neuroepithelium of the central nervous system, (2) the attachment sites of muscle to epidermis, and (3) the cardioblasts of the dorsal tube. The expression of SLIT in the cardioblasts as they meet and form the lumen of the dorsal tube may be of general interest given that, in vertebrate tissue culture, the extracellular matrix has been shown to be involved in endothelial cell alignment and the induction of capillary tube formation. (Ingber, D. E., and Folkman, J. (1989). How Does the Extracellular Matrix Control Capillary Morphogenesis? Cell58, 803-805). This process is one of the best characterized morphogenetic processes in vitro and has allowed for an analysis of the molecular mechanisms by which ECM molecules, specifically collagen, laminin, and fibronectin are able to control capillary morphogenesis (Grant, D. S., Tashiro, K., Segui-Real, B., Yamada, Y., Martin, G. R., Kleinman, H. K. (1989). Two different laminin domains mediate the differentiation of human endothelial cells into capillary-like structures in vitro. Cell 58, 933-943).
In Drosophila, the larval heart or dorsal vessel is derived from two longitudinal rows of mesodermal cells termed cardioblasts. When these cells meet following dorsal closure along the midline, only their dorsomedial and ventromedial surfaces contact, the space between forming the lumen of the dorsal vessel (Poulson, E. F. (1950). Histogenesis, Organogenesis, and Differentiation in the Embryo of Drosophila Melanogaster Meigen. In Biology of Drosophila, M. Demerec, ed. (New York: Wiley), 168-274): Hartenstein, V., and Campos-Ortega, J. A. (1985). The embryonic development of Drosophila melanogaster. Springer-Verlag; Berlin). SLIT is expressed in the developing cardioblasts during the time they come together. Confocal microscopic imaging clearly shows the SLIT protein to be concentrated at the point of contact between the cardioblasts as they come together and form the lumen of the larval heart. Given SLIT's unique structural characteristics, its homology to ECM binding proteins, and the role of these ECM proteins in vessel formation, an analysis of SLIT's role in developing cardioblasts and its possible interactions with other proteins expressed in these tissues during larval heart formation would serve as a useful in vivo model for the study of the angiogenic process.

Confocal microscopy shows the SLIT protein to be tightly localized to the points of muscle attachment to the epidermis. This localization is consistent with SLIT functioning as an ECM molecule, and suggests its involvement in adhesive events. The muscle attachment sites are known sites of ECM deposition (Newman, S. M. Jr., and Wright, R. F. (1981). A histological and Ultrastructural Analysis of Development Defects Produced by the Mutation, lethal (1) myospheroid, in Drosophila melanogaster. Dev. Bio. 86, 393-402), and the position-specific integrins have been shown to be localized here (Leptin, M., Bogaert, T., Lehmann, R., and Wilcox, M (1989). The Function of PS Integrins during Drosophila
Embryogenesis. Cell 56, 401-408). Hence, a role for SLIT in adhesive-mediated events such as muscle attachment and axonal outgrowth is supported both by its structure and its expression pattern. The potential for two variants of the SLIT protein raises the possibility that these roles are mediated by functionally distinct forms of the protein.

Tissue culture studies have demonstrated that growth cones adhere to and extend neurites onto ECM molecules such as laminin and fibronectin (Sanes et al., 1989 supra) and that the direction and rate of axonal growth are dependent on these axon matrix interactions (Rutishauser, U., and Jessell, T. M. (1988). Cell adhesion molecules in vertebrate neural development. Pysiol. Rev. 68, 819-857). Given SLIT's homology to the laminin binding protein biglycan, it is noted with interest that laminin is expressed on glial surfaces and along the pathways axons follow in the establishment of the commissural and longitudinal axonal tracts in Drosophila (Montell, D. J. and Goodman, C. S. (1989). Drosophila laminin: sequence of B2 subunit and expression of all three subunits during embryogenesis. J. Cell. Bio. 109, 2441-2453). The possibility that SLIT binds to matrix materials suggests that its presence on growing axons could influence their interactions with ECM proteins. The ability of axons to fasciculate on one another in all SLIT mutants indicates that SLIT is not necessary for axon-axon fasciculation. However, the combination of Flank-LRR-Flank tandem EGF and single EGF motifs in a protein with SLIT's unique embryonic distribution could allow for the formation of a "molecular-bridge" between axonally associated receptors and ECM molecules. Prompted by the information on SLIT's structure, its expression in glial cells and its presence on axons which extend along these cells, a mechanism whereby glial cells can influence an axon's future behavior is as follows:
(1) Glial cells secrete multi-functional molecules (TAGONS) into the endoneurial basal lamina. These TAGONS have the ability to attach to specific axonal receptors as well as to specific ECM components.

(2) Passing axons carrying receptors for these proteins pick them up from the glial cell surroundings.

(3) Depending on the proteins associated with them, axons are able to respond to cues and interact with molecules in the ECM.

   SLIT is one of the TAGONS.

Therapeutics

The SLIT protein is a unique extracellular matrix protein with applications in nerve regeneration, angiogenesis, and control of neoplasms. SLIT is involved in the development of axon pathways.

The SLIT protein is involved in the development and maintenance of the central nervous system, including the process of glial cell differentiation and neuronal outgrowth. The SLIT protein also plays an inductive role in vessel formation.

The SLIT protein facilitates interactions between cell surface receptors and extracellular matrix molecules, hence providing for a novel molecular link between a cell's environment and genes (including known oncogenes) involved in cellular proliferation and differentiation.

The SLIT protein is involved in the development of cell specificity and the process of neuronal outgrowth.
The SLIT protein molecule can be a therapeutic especially for the repair of damaged neuronal tissue, either alone or in combination with neuronal growth factors (NGF) or other extracellular molecules, and it is useful in nerve repair and tissue regeneration.

The SLIT protein defines a new and novel set of molecules (TAGONS) which play a key role in axon outgrowth and pathfinding. The SLIT protein is thus involved in neurogenesis, axonogenesis, cell differentiation, organ formation and vessel formation and also in muscle attachment.

The SLIT protein can be utilized as a nerve regenerative in neurodegenerative diseases, e.g., it can be utilized as a therapeutic for the following conditions: Alzheimer's disease, spinal cord injuries, brain injuries, crushed optic nerve, nerve damage, amyotrophic lateral sclerosis (ALS), crushed nerves, diabetes-caused nerve damage, facial nerve damage resulting in facial paralysis, Parkinson's disease, strokes, epilepsy, multiple sclerosis, paraplegia and retinal degeneration.

The SLIT proteins of the invention can be formulated into pharmaceutically acceptable preparations with parenterally acceptable vehicles and excipients in accordance with procedures known in the art.

The pharmaceutical preparations of this invention, suitable for parenteral administration, may conveniently comprise a sterile lyophilized preparation of the protein which may be reconstituted by addition of sterile solution to produce solutions, preferably isotonic with the blood of the recipient. The preparation may be presented in unit or multi-dose containers, e.g., in sealed ampoules or vials.

The pharmaceutical preparation may in some instances by orally administered in the form of pills, tablets or capsules.
In use, purified SLIT protein is administered to a mammal, e.g., a human, for treatment in a manner appropriate to the indication. Administration may be by injection, continuous infusion, sustained release from implants (such implants may take the form of a biodegradable plastic or resin having the therapeutic imbedded therein), or other suitable technique. Where the SLIT protein is administered as an aid to wound healing, it will typically be applied topically to the site of the injury, for example, in conjunction with a wound dressing. Therapeutically-effective dosage levels are determined by initiating treatment at higher dosage levels and reducing the amounts of the SLIT protein administered until the condition sought to be alleviated, e.g., wound healing including, but not limited to, neuronal trauma, is no longer achieved. Generally, therapeutic dosages will range from about 0.1 to 1000 ng per g body weight, preferably 1-100 ng/kg. Dosage will vary based on several factors including the weight of the patient and the severity of the condition or ailment. Typically, the SLIT protein will be administered in the form of a composition comprising purified protein in conjunction with physiologically acceptable carriers, excipients or diluents. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents.

The present invention also envisages methods for the treatment of animals in need thereof, such animals preferably being mammals, and most preferably being human beings. The treatment will tend to comprise administration of non-toxic formulations described above in the appropriate manner and in suitable doses. SLIT is involved in the development of axon pathways. Alone, or possibly in combination with neuronal growth factors, SLIT is expected to find use in nerve repair and tissue regeneration. The involvement of the SLIT protein
in organ/vessel formation will lead to either direct or indirect therapeutic applications in the control of neoplasms.

**Diagnostics**

The SLIT proteins according to the present invention and antibodies raised thereto can be employed in immunoassays. Such antibodies can be polyclonal antibodies or monoclonal antibodies.

The detection of SLIT in the bloodstream of a patient is important because such is an indication of an abnormal condition, since SLIT does not appear in the blood of a normal mammal. The presence of SLIT in one's blood may be, for example, an indication of a cancerous condition.

A monoclonal antibody can be prepared according to known methods, for example by the procedures of immunization, cell fusion, screening, and cloning, using the procedures of G. Kohler and C. Milstein (1975), Nature (Lond.), 256, 495.

In selection of the animal to be immunized for production of a monoclonal antibody, the animal species and the immune response to the antigen are important. Generally speaking, stable antibody-producing hybridomas will be frequently formed with good efficiency when the spleen cells to be used and myeloma are of the same animal species. Particularly preferred is the use of BALB/c mice. Preferred myeloma cell species include \( \text{P3}\cdot\text{X63}\cdot\text{Ag8(X63)} \), \( \text{P3}\cdot\text{NS}-1/1\cdot\text{Ag4}\cdot1(\text{NS}-1) \), \( \text{SP2/0}\cdot\text{Ag14(SP-2)} \) and \( \text{FO} \).

The antibody, protein or sample in the immunoassays of the invention may be immobilized to a support. Known immobilization techniques and materials can be employed. Examples of immobilization methods include the
physical adsorption method, the ion bonding method, the covalent bonding method, the support crosslinking method, the support-less crosslinking method, and the inclusion method.

The support may be one generally used, and the choice is not particularly limited. Selection of the support depends on the properties of the material to be immobilized, but it is also necessary to consider the size of particules, the surface area in the three-dimensional network structure, the ratio of hydrophilic sites to hydrophobic sites, chemical composition, strength to pressure, etc. of the support. Typical examples of the support include polysaccharide derivatives such as cellulose, dextran, or agarose; synthetic polymers such as polyacrylamide gel, or polystyrene resin; and inorganic materials such as porous glass, or metal oxide.

With the physical adsorption method, where the material is immobilized by physical adsorption onto a water-insoluble support, examples of particularly preferred supports include inorganic substances such as activated charcoal, porous glass, acidic white clay, bleached clay, kaolinite, alumina, silica gel, bentonite, hydroxyapatite, calcium phosphate, metal oxide, or ceramic; a natural polymer such as starch or gluten; or a porous synthetic resin. Adsorption hydrophobically onto a support having hydrophobic groups such as butyl- or hexyl-
"SEPHADEX" is also possible.

With the ion bonding method, where the material is immobilized by binding ionically to a water-insoluble support having ion exchange groups, particularly preferred examples of the support include polysaccharides having ion exchange groups such as DEAE-"SEPHADEX" or synthetic polymer derivatives such as ion exchange resins.
With the covalent bonding method, where the material is immobilized by covalent bonding to a water-insoluble support, examples of particularly preferred supports include those having amino, carboxyl, sulfhydryl, hydroxy, imidazole or phenol groups which are functional groups reactive for instance with diazonium salts, acid azides, isocyanates, or active type alkyl halides.

With the support crosslinking method, where the material is immobilized to the support by covalent binding with the use of a crosslinking reagent such as glutaraldehyde, examples of particularly preferred supports include water-insoluble supports having amino groups, such as AE-cellulose, DEAE-cellulose, partially deacetylated chitin, or aminoalkylated porous glass.

With the support-less crosslinking method, where immobilization is effected by crosslinking materials with a reagent having two or more functional groups, no support is particularly required. Examples of preferred crosslinking reagents include glutaraldehyde (forming a Shiff's base), an isocyanic acid derivative (forming a peptide), N,N'-ethylenebismaleimide, bisdiazobenzidine (for diazo coupling), or N,N'-polymethylenebisisoacacetamide (alkylating agent). The material which participates in the crosslinking reaction needs a suitable functional group at the N-end, such as an amino group, phenol group, sulfhydryl group or imidazole group.

With the inclusion method, the method may be classified into the lattice type in which materials to be immobilized are incorporated into fine lattices of polymeric gels, and the microcapsule type in which the antibodies or antigens are coated with semipermeable polymeric films. Examples of preferred supports in the case of the lattice type include polymeric compounds, for example, synthetic polymeric
substances such as polyacrylamide gel, polyvinyl alcohol, or photocurable resin; and natural polymeric substances such as starch, konjak powder, gelatin, alginic acid, or carrageenan. In the case of the microcapsule type, various techniques are possible. When the interfacial polymerization method is used, namely the method in which the antibody is coated by utilizing the principle of polymerizing a hydrophilic monomer and a hydrophobic monomer at the interface therebetween, a nylon film based on hexamethylenediamine and sebacoyl chloride can be employed. When the drying-in-liquid method is used, namely the method in which an antibody solution is dispersed in a polymeric compound solution dissolved in an organic solvent to form an emulsion and then transferred into an aqueous solution followed by drying, thereby coating the antibody, examples of preferred supports include polymeric substances such as ethyl cellulose or polystyrene. When the phase separation method is used, namely the method in which a polymeric compound is dissolved in an organic solvent immiscible with water, an antibody is dispersed in the solution to prepare an emulsion, then a non-solvent which causes phase separation is gradually added under stirring, whereby a concentrated solution of the polymeric compound encloses the antibody droplets therearound, and subsequently the polymeric compound is precipitated to form a film which covers the antibody, is used, the above-mentioned polymeric compounds can be employed.

Labels for use in the present invention include substances which have a detectable physical, chemical or electrical property. When a detectable labeling substance is introduced, it can be linked directly such as by covalent bonds or can be linked indirectly such as by incorporation of the ultimately detectable substance in a microcapsule or liposome.
Labelling materials have been well-developed in the field of immunoassays and in general almost any label useful in such methods can be applied to the present invention. Particularly useful are enzymatically active groups, such as enzymes (see Clin. Chem., (1976) 22:1232, U.S. Reissue Pat. No. 31,006, and UK Pat. 2,019,408), enzyme substrates (see U.S. Pat. No. 4,492,751), coenzymes (see U.S. Pat. Nos. 4,230,797 and 4,238,565), and enzyme inhibitors (see U.S. Pat. No. 4,134,792): fluorescers (see Clin. Chem., (1979) 25:353); chromophores; luminescers such as chemiluminescers and bioluminescers (see U.S. Pat. 4,380,580); specifically bindable ligands such as biotin (see European Pat. Spec. 63,879) or a hapten (see PCT Publ. 83-2286); and radioisotopes such as $^3$H, $^{35}$S, $^{32}$P, $^{125}$I, and $^{14}$C. Such labels are detected on the basis of their own physical properties (e.g., fluorescers, chromophores and radioisotopes) or their reactive or binding properties (e.g., ligands, enzymes, substrates, coenzymes and inhibitors). For example, a cofactor-labeled species can be detected by adding the enzyme (or enzyme where a cycling system is used) for which the label is a cofactor and a substrate or substrates for the enzyme. Such detectable molecule can be some molecule with a measurable physical property (e.g., fluorescence or absorbance) or a participant in an enzyme reaction (e.g., see above list). For example, one can use an enzyme which acts upon a substrate to generate a product with a measurable physical property.

Any convenient immunoassay technique can be employed in the present invention including, for example, enzyme-linked immunoassay, radioimmunoassay (RIA), immunofluorescence and the use of dyes.

In enzyme linked immunoassays, an enzyme is conjugated to an antibody or antigen and the enzyme activity is measured as
a quantitative label. A particularly preferred enzyme linked immunooassay is enzyme-linked immunosorbent assay (ELISA).

The enzyme may be any of the enzymes generally used in enzyme immunoassay, including maleate dehydrogenase, glucose-6-phosphoric acid dehydrogenase, glucose oxidase, peroxidase, acetylcholine esterase, alkali phosphatase, glucoamylase, lysozyme, β-D-galactosidase, etc., preferably peroxidase, alkali phosphatase or β-D-galactosidase or horseradish peroxidase.

Immunofluorescence utilizes fluorescent dyes such as fluorescein isothiocyanate or rhodamine.

The detection of nucleic acids involves hybridization conditions and techniques that are known in the art. The principle for the hybridization test is as follows:

Two DNAs are heated to denature them completely, with separation of strands. When they are mixed and slowly cooled, complementary DNAs of each species will find each other and reanneal to form normal duplexes. But if the two DNAs have significant sequence homology, they will tend to form partial duplexes or hybrids with each other. The greater the sequence homology between two DNAs, the greater the number of hybrids formed. Hybrid formation can be measured by different procedures, e.g., chromatography or density-gradient centrifugation. Usually one of the DNAs is labeled with a radioactive isotope to simplify the measurements.

The SLIT nucleic acid molecule according to the present invention can be used as a gene probe, i.e., a nucleic acid molecule that can be used to detect, by complementary base-pairing, another nucleic acid molecule that has a complementary or homologous sequence. The probe is invariably
labeled, e.g., Nick translation, Biotin, to allow autoradiographic or enzymatic detection of the hybridization reaction.

The Southern transfer method can be utilized in the present invention. The Southern transfer procedure (developed by Edwin Southern and sometimes called blotting), a method for performing hybridization to particular DNA segments, avoids the necessity of purifying the DNA fragments with restriction endonucleases.

At present the best way to separate DNA fragments from one another is by electrophoresis through agarose gels. A specific fragment can be isolated by cutting out of a gel a portion that contains the fragment of interest. A variety of procedures, most of which are cumbersome and tedious, are available for recovering the DNA molecule from the gel. If hybridization is to be performed, the fragment must be bound to a nitrocellulose filter. In the Southern transfer technique a collection of fragments is handled in such a way that all fragments are transferred from a gel to a sheet of nitrocellulose in a single step, significantly simplifying the entire process.

The Southern transfer technique is carried out as follows: DNA is enzymatically fragmented and then electrophoresed through an agarose gel. Following electrophoresis the gel is soaked in a denaturing solution (usually NaOH), so that all DNA in the gel is converted to single-stranded DNA, which is needed for hybridization. A large sheet of nitrocellulose paper is placed on top of several sheets of ordinary filter paper; the gel, which is typically in the form of a broad flat slab, is then placed on the nitrocellulose filter and covered with a glass plate to prevent drying. A weight is then placed on the top of the stack and the liquid is squeezed out of the
gel. The liquid passes downward through the nitrocellulose filter. Denatured DNA binds tightly to nitrocellulose; the remaining liquid passes through the nitrocellulose and is absorbed by the filter paper. DNA molecules do not diffuse very much, so that if the gel and the nitrocellulose are in firm contact, the positions of the DNA molecules on the filter are identical to their positions in the gel. The nitrocellulose filter is then dried in vacuum, which insures that the DNA remains on the filter during the hybridization step. The dried filter is then moistened with a very small volume of a solution of $^{32}$P-labeled RNA, placed in a tight-fitting plastic bag to prevent drying, and held at a temperature suitable for renaturation (usually for 16-24 hours). The filter is then removed, washed to remove unbound radioactive molecules, dried, and auto radiographed with x-ray film. The blackened positions of the film indicate the locations of the DNA molecules whose DNA base sequences are complementary to the sequences of the added radioactive molecules.

For the most part, the probe will be labeled with an atom or inorganic radical, most commonly using radionuclides, but also perhaps heavy metals.

Conveniently, a radioactive label may be employed. Radioactive labels include $^{32}$P, $^3$H, $^{14}$C, or the like. Any radioactive label may be employed which provides for an adequate signal and has sufficient half-life. Other labels include ligands, which can serve as a specific binding member to a labeled antibody, fluorescers, chemiluminescers, enzymes, antibodies which can serve as a specific binding pair member for a labeled ligand, and the like. A wide variety of labels have been employed in immunoassays, as discussed hereinabove, which can readily be employed in the present hybridization assay. The choice of the label will be governed by the effect
of the label on the rate of hybridization and binding of the probe to the genetic nucleic acid, e.g., DNA. It will be necessary that the label provide sufficient sensitivity to detect the amount of DNA available for hybridization. Other considerations will be ease of synthesis of the probe, readily available instrumentation, ability to automate, convenience, and the like.

The manner in which the label is bound to the probe will vary depending upon the nature of the label. For a radioactive label, a wide variety of techniques can be employed. Commonly employed is Nick translation with an α-\( ^{32} \text{P}\)-dNTP or terminal phosphate hydrolysis with alkaline phosphatase followed by labeling with radioactive \( ^{32} \text{P} \) employing \( \gamma - ^{32} \text{P} \)-NTP and T4 polynucleotide kinase. Alternatively, nucleotides can be synthesized where one or more of the elements present are replaced with a radioactive isotope, e.g., hydrogen with tritium. If desired, complementary labeled strands can be used as probes to enhance the concentration of hybridized label.

Where other radionuclide labels are involved, various linking groups can be employed. A terminal hydroxyl can be esterified, with inorganic acids, e.g., \( ^{32} \text{P} \) phosphate, or \( ^{14} \text{C} \) organic acids, or else esterified to provide linking groups to the label. Alternatively, intermediate bases may be substituted with activatable linking groups which can then be linked to a label.

Ligands and antiligands may be varied widely. Where a ligand has a natural receptor, namely ligands such as biotin, thyroxine, and cortisol, these ligands can be used in conjunction with labeled naturally occurring receptors. Alternatively, any compound can be used, either haptenic or antigenic, in combination with an antibody.
Enzymes of interest as labels will primarily be hydrolases, particularly esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescers include luciferin, and 2,3-dihydrophthalazinediones, e.g., luminol.

The probe can employed for hybridizing to a gene affixed to a water insoluble porous support. The single stranded nucleic acid is affixed. Depending upon the source of the nucleic acid, the manner in which the nucleic acid is affixed to the support may vary.

A clinical isolate or specimen can be spotted or spread onto a filter to provide a plurality of individual portions. The filter is an inert porous solid support, e.g., nitrocellulose. The clinical isolate can be blood or another bodily fluid from a patient, e.g., a human patient. Conveniently, a microfilter is employed, which inhibits the passage of the cells through the filter.

The cells are then treated to liberate their DNA. Lysis conditions are devised such that the cells do not migrate and their DNA remains affixed in place on the surface where they were situated. The lysing and DNA denaturing, as well as the subsequent washings, can be achieved by placing the filter containing the cells isolate side up, onto a bibulous support saturated with an appropriate solution for a sufficient time to lyse the cells and denature the DNA. For lysing, chemical lysing will conveniently be employed, usually dilute aqueous alkali, e.g., 0.1 to 1 M NaOH. The alkali will also serve to denature the DNA. Other denaturation agents include, elevated temperatures, organic reagents, e.g., alcohols, amides,
amines, ureas, phenols and sulfoxides or certain inorganic ions, e.g., thiocyanate and perchlorate.

After denaturation, the filter is washed in an aqueous buffered solution, generally at a pH of about 6 to 8, usually 7. Of the many different buffers that may be used, tris is an example. One or more washings may be involved, conveniently using the same procedure as employed for the lysing and denaturation.

After the lysing, denaturing and washes have been accomplished, the DNA spotted filter is dried at an elevated temperature, generally from about 50° to 70°C. The DNA is now fixed in position and can be assayed with the probe when convenient. This fixing of the DNA for later processing has great value for the use of this technique in field studies, remote from laboratory facilities.

Hybridization may now be accomplished. The filter is incubated at a mildly elevated temperature for a sufficient time with the hybridization solution without the probe to thoroughly wet the filter. Various hybridization solution may be employed, comprising from about 20 to 60 volume, preferably 30, percent of an inert polar organic solvent. A common hybridization solution employs about 50% formamide, about 0.5 to 1 M sodium chloride, about 0.05 to 0.1 M sodium citrate, about 0.05 to 0.2% sodium dodecylsulfate, and minor amounts of EDTA, ficoll (about 300-500 kdaltons), polyvinylpyrrolidone, (about 250-500 kdaltons) and serum albumin. Also included in the hybridization solution will generally be from about 0.5 to 5 mg/ml of sonicated denatured DNA, e.g., calf thymus of salmon sperm; and optionally from about 0.5 to 2% wt/vol. glycine. Other additives may also be included, such as dextran sulfate of from about 100 to 1,000 kdaltons and in an amount
of from about 8 to 15 weight percent of the hybridization solution.


The amount of labeled probe which is present in the hybridization solution will vary widely, depending upon the nature of the label, the amount of the labeled probe which can reasonably bind to the filter, and the stringency of the hybridization. Generally, substantial excesses over the stoichiometric amount of the probe will be employed to enhance the rate of binding of the probe to the fixed DNA.

Various degrees of stringency of hybridization will be employed. The more severe the conditions, the greater the complementarity that is required for hybridization between the probe and the ssDNA (single stranded DNA) for duplex formation. Severity can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Conveniently, the stringency of hybridization is varied by changing the polarity of the reactant solution by manipulating the concentration of formamide in the range of 20% to 50%. Temperatures employed will normally be in the range of about 20° to 80°C, usually 30° to 75°C.

After the filter has been contacted with a hybridization solution at a moderate temperature for an extended period of time, the filter is then introduced into a second solution having analogous concentrations of sodium chloride, sodium citrate and sodium dodecylsulfate as provided in the
hybridization solution. The time for which the filter is maintained in the second solution may vary five minutes to three hours or more. The second solution determines the stringency, dissolving cross duplex and short complementary sequences. After rinsing the filter at room temperature with dilute sodium citrate-sodium chloride solution, the filter may now be assayed for the presence of duplexes in accordance with the nature of the label. Where the label is radioactive, the filter is dried and exposed to X-ray film.

**Expression**

The nucleotide sequences of the invention are preferably sequences of DNA. Such sequences may be used alone, for example as probes, but it is generally preferred that they form part of an expression system. Thus, it is preferred that the DNA sequence form part of a vector useful in an expression system.

The general nature of vectors for use in accordance with the present invention is not crucial to the invention. In general, suitable vectors and expression vectors and constructions therefor will be apparent to those skilled in the art.

Suitable expression vectors may be based on phages or plasmids, both of which are generally host-specific, although these can be engineered for other hosts. Other suitable vectors include cosmids and retroviruses, and any other vehicles, which may or may not be specific for a given system. Again, control sequences, such as recognition, promoter, operator, inducer, terminator and other sequences essential and/or useful in the regulation of expression, will be readily apparent to those skilled in the art, and may be associated with the natural SLIT protein sequence or with the vector.
used, or may be derived from any other source as suitable. The vectors may be modified or engineered in any suitable manner.

In general, there are a number of methods which can be used to produce the peptide and nucleotide sequences of the invention. One straightforward method is simply to synthesize the appropriate nucleotide sequence, insert it into a suitable expression plasmid, transform a suitable host, culture the host, and obtain the SLIT protein of the invention by any suitable means, such as sonication and centrifugation.

Alternatively, fragments can be obtained by digestion with the relevant restriction enzymes, and a suitable oligonucleotide ligated to the 5'-end coding for missing amino acids. The resulting cDNA can then be used as above.

Other suitable methods will be apparent to those skilled in the art.

It will be appreciated that the fragment encoding the SLIT protein of the invention may easily be inserted into any suitable vector for any purpose desired. Suitable vectors may be selected as a matter of course by those skilled in the art according to the expression system desired.

By transforming E. coli with the plasmid obtained, selecting the transformant with ampicillin or by other suitable means, and adding tryptophan or other suitable promoter inducer such as indoleacrylic acid, the desired protein may be expressed. The extent of expression may be analyzed by SDS polyacrylamide gel electrophoresis - SDS-PAGE (Nature, (1970), 227, pp.680-685).
It will also be appreciated that, where another vector is used, for example, it will be equally acceptable to employ a different selection marker or markers, or an alternative method of selection, and/or to use any suitable promoter as required or convenient.

After cultivation, the transformant cells are suitably collected, disrupted, for example, sonicated, and spun-down. Disruption may also be by such techniques as enzymic digestion, using, for example, cellulase, or by shaking with an agent such as glass beads, but methods such as sonication are generally preferred, as no additions are necessary. The activity of the supernatant may be assayed and the amount of the SLIT protein measured by SDS-PAGE, for example, allowing the specific activity to be calculated.

Conventional protein purification is suitable to obtain the expression product.

Where not specifically described herein, methods for growing and transforming cultures etc. are usefully illustrated in, for example, Maniatis (Molecular Cloning, A Laboratory Notebook, Maniatis et al. [Ed's], Cold Spring Harbor Labs, NY).

Cultures useful for the production of the SLIT protein of the invention may suitably be cultures of any living cells, and may vary from prokaryotic expression systems up to eukaryotic expression systems. One preferred prokaryotic system is that of *E. coli*, owing to its ease of manipulation. However, in general terms, it is preferable to express proteins intended for use in the human body in higher systems, especially mammalian cell lines. A currently preferred such system is the Chinese Hamster Ovary (CHO) cell line. Although this system tends not to be as easy to use as the *E. coli*
system, its advantage lies in the processing of the protein after primary synthesis. *E. coli*, for example, does not have the equipment to glycosylate mammalian proteins, and it is preferred to glycosylate such proteins where possible, if for no other reason than that the natural proteins are glycosylated. In certain cases, glycosylation may be of no assistance whatever, and may even hinder the protein. In the present instance, glycosylation appears to serve little purpose.

Other expression systems which may be employed include streptomyces, for example, and yeasts, such as *Saccharomyces* spp., especially *S. cerevisiae*. With current progress in research, other systems are becoming available and there is no effective limit on which system is used, provided that it is suitable. The same systems may also be used to amplify the genetic material, but it is generally convenient or use *E. coli* for this purpose where only proliferation of the DNA is required.

**Equivalents, Variants and Mutants**

In general, it will be appreciated that the activity of any given protein is dependent upon certain conserved regions of the molecule, while other regions have little importance associated with their particular sequence, and may be virtually or completely redundant. Accordingly, the present invention also includes any equivalents, variants and mutants on the sequence which still show substantial activity. Such variants and mutants include, for example, deletions, insertions, repeats and type-substitutions (e.g., substituting one hydrophilic residue for another, but not strongly hydrophilic for strongly hydrophobic as a rule). Small changes will be generally have little effect on activity, unless they are an essential part of the molecule, and may be
a side-product of genetic manipulation, for example, when generating extra restriction sites, if such is desired.

It will be appreciated that the coding sequence may be modified in any manner desired, provided that there is no adverse effect on activity. Spot mutations and other changes may be effected to add or delete restriction sites, for example, to otherwise assist in genetic manipulation/expression, or to enhance or otherwise conveniently modify the SLIT molecule.

As used herein, the term a "adverse effect" means any effect on activity, or as otherwise used, which renders the molecule only as effective as, or less effective as, the naturally occurring SLIT protein.

If desired, the carboxy terminal group or other carboxyl groups of the SLIT protein may be substituted or modified in any manner apparent to those skilled in the art. Such substitutions may include the formation of salts and esters, for example, or any other substitution as appropriate. Modification may include the deletion of one or more C-terminal amino acid residues, partially or entirely, provided that this has no adverse effect on activity. Deletion of the terminal carboxyl group may be useful in preventing undesirable reactions, which purpose may also be served by the use of an appropriate protecting group, for example. Modification may also include replacement of one or more of the residues with any other suitable residue, and such replacement may either be 1:1 or any other suitable ratio.

Modifications but, more especially, substitutions to the C-terminal may either be temporary or permanent, as with modifications and substitutions to the SLIT protein molecule as a whole. Thus, a C-terminal esterified SLIT protein may be
de-esterified in vivo, either at or before reaching the target site. Likewise, the SLIT protein may be specifically modified, particularly by deletion or substitution, so as to be inactive until the target is reached, whereon activation may be internal, by enzymatic cleavage or addition, for example, or external, such as by irradiation to activate a sensitive group.

In general, it will be appreciated that the entire molecule may be substituted or modified within wide limits. Thus, for example, it will be apparent that the SLIT protein of the invention may be heavily glycosylated without adversely affecting activity. The present invention envisages both glycosylated and unglycosylated SLIT protein of the invention as being useful, as well as any state in between.

Many substitutions, additions, and the like may be effected, and the only limitation is that activity not be adversely affected. In general, an adverse effect on activity is only likely if the 3-D (tertiary) structure of the SLIT protein is seriously affected, or if an active site is somehow affected (reducing electronegativity/hydrophilicity, blocking etc.).

If it is desired to glycosylate the SLIT protein molecule selectively, rather than randomly as would be achieved by direct chemical addition, this can be achieved best by a eukaryotic, especially mammalian, system. This may either comprise a eukaryotic expression system, or treatment of the product with a suitable enzyme system in vitro, both of which are known in the art.

Selective substitution on the molecule will not generally be facile. For example, to modify only the C-terminal carboxy, it would most likely be necessary to block any other
groups likely to be modified by the same treatment. Universal modification of a particular type of group may be acceptable, such as esterification, but it is usually acceptable and, moreover, practical to use the unmodified expression product. However, selective modification is particularly achieved by appropriate selection of expression system and/or suitable modification of the coding sequence.

Suitable substitutions, additions and the like may be effected as desired to assist in formulation, for example, or may be a product of any expression system employed.

With reference to the peptide sequences disclosed herein, the term "equivalent" is used in the sense of the preceding description, that is to say, equivalents in the sense of sequences having substitutions at the C- or N-terminals, or anywhere else, including salts and esters, and glycosylated sequences. The term "mutants" is used with reference to deletions, insertions, inversions and replacements of amino acid residues in the sequence which do not adversely affect activity. "Variant" is used in relation to other naturally occurring SLIT proteins which may be discovered from time to time and which share essentially as shown in the sequences herein, but which vary therefrom in a manner to be expected within metazoan organisms. Within this definition lies allelic variation. The term "precursor" includes such molecules as those having leader sequences or substitutions which may or may not affect activity, but which are no longer present when the SLIT protein is active, whether the effect was negated before or at the target site.

The present invention also provides nucleotide sequences encoding all or part of the SLIT proteins of the invention. As will be apparent from the foregoing, there is little restriction on the sequence, whether it be DNA or RNA. A gene
encoding the SLIT proteins of the invention may easily be reverse-engineered by one skilled in the art from the sequences given herein together with the information provided herein.

It will be appreciated that any one given reverse-engineered sequence will not necessarily hybridize well, or at all, with any given complementary sequence reverse-engineered from the same peptide, owing to the degeneracy of the genetic code. This is a factor common in the calculations of those skilled in the art, and the degeneracy of any given sequence is frequently so broad as to make it extremely difficult to synthesize even a short complementary oligonucleotide sequence to serve as a probe for the naturally occurring oligonucleotide sequence.

The degeneracy of the code is such that, for example, there may be four, or more, possible codons for frequently occurring amino acids. Accordingly, therefore, it can be seen that the number of possible coding sequences for any given peptide can increase exponentially with the number of residues. As such, it will be appreciated that the number of possible coding sequences for the SLIT protein of the invention may have several figures, with little to choose between any of that number. However, it may be desirable to balance the GC ratio according to the expression system concerned, and other factors may need to be taken into account which may affect the choice of coding sequence.

The invention is now described with reference to the following non-limiting examples.

Example 1: Cloning By Transposon Tagging
slit^{61} and slit^{119} were created by germline transformation with the enhancer trap construct P-lacZ (Bier et al., (1989)).
Searching for pattern and mutation in the Drosophila genome with a P-lacZ vector. Genes & Dev. 3, 1273-1287) and slit\textsuperscript{158} was made using P-lA2B (Bellen, H. J., O'Kane, C. J., Wilson, C., Grossniklaus, U., Pearson, R. K., and Gehring, W. J. (1989). P-element-mediated enhancer detection: a versatile method to study development in Drosophila. Gen. & Dev. 3, 1288-1300). Other SLIT alleles are as described in Rothberg et al., 1988, supra slit\textsuperscript{175} exhibit some ectopic $\beta$-galactosidase expression, while slit$^{81}$ and slit$^{119}$ (likely the result of the same insertion event) have levels of midline expression lower than levels in slit$^{158}$. Lines 8-7 and 242 function as cell autonomous markers for the midline neuroepithelium and contain the PZ and HZ enhancer trap constructs which use the P-element and ftz promoters, respectively, to drive $\beta$-galactosidase expression. Line 5704 expresses $\beta$-galactosidase from the ftz promoter in the MP2 cells (Hiromi, Y., Kuroiwa, A., and Gehring, W. J. (1985). Control elements of the Drosophila segmentation gene fushi tarazu. Cell 43,603-613). Lines 8-7, 242 and 5704 were made homozygous in slit$^{1610}$/CyO flies to characterize the development of the midline in slit$^{1610}$/slit$^{1610}$ embryos.

Example 2: Isolation of cDNA and Genomic Clones

Isolation of the initial slit cDNA clones was described in Rothberg et al., (1988), supra. Both the polymerase chain reaction (PCR) (Saiki R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R. Horn, G. T., Mullis, K. B., and Erlich, H. A. 1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239, 487-491) and standard library screening methods (Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory) were employed to extend this analysis. A cDNA clone representing the 5' most 2.4kb of sequence (ka2.4) was isolated from the larval library of Poole, S. J. Kauvar,

Two forms of the SLIT message were evident differing by 33 nucleotides, when restriction fragments from the larger class (B52-1 and B52-2) were compared with those from the smaller class (B52-5). Primer pairs covering adjacent segments of the coding region were utilized in the PCR to screen embryonic cDNA libraries (Poole et al., 1985, supra; Brown and Kafatos, 1988, supra) for the presence of multiple cDNA forms. Two classes already represented by B52-1,2 and B52-5 were generated. Genomic and cDNA sequencing indicates the transcripts consists of an approximately 314 bp 5' untranslated leader sequence, followed by either a 4407 or 4440 bp ORF depending on the splice form and a 4 kb untranslated 3' end. EcoRI cDNA fragments representing the entire transcription unit were aligned with genomic sequences by Southern analysis.

Example 3: Subcloning, Sequencing, Localization of Transposon insertion Sites

The relevant regions from phage, plasmid and PCR-generated cDNAs were subcloned into Bluescript (Stratagene) or M13mp18/19 vector. Single-stranded templates were sequenced directly or subjected to deletions by T4 polymerase (International Biotechnologies Corp.). Chain termination sequencing (Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463-5467) used Sequenase v2.0 (United States Biochemical Corp.). dITP was employed where sequence was ambiguous and synthetic
oligonucleotides were used as primers to fill any gaps in the nested deletions. The use of gene-specific and P-element inverted repeat-specific primers to isolate genomic DNA using PCR was previously described in Ballinger, D. G., Benzer, S., (1990). Targeted gene mutations in Drosophila. Proc. Natl. Acad. Sci. USA 86, 9402-9406. Sequences from the 31 bp inverted P-element repeat (O'Hare, K., and Rubin, G.M. (1983). Structure of P Transposable Elements and Their Sites of Insertion and Excision in the Drosophila melanogaster Genome. Cell 34, 25-35) and from the 5' region of the SLIT transcript were used as primers. Sequencing of PCR products was performed on a Dupont Genesis 2000 sequencing machine after the generation of single-stranded DNA by asymmetric PCR and the removal of excess primers with Sepharose S-200 spin columns. Sequence analysis was accomplished with MacVector (International Biotechnologies Inc.) on a Macintosh II. Database searches and sequence comparisons were conducted using the FASTA package (Pearson, W. R., and Lipman, D. J.. (1988). Improved tools for biological sequence comparison. Proc. Nat. Acad. Sci. USA 85, 2444-2448) with version 23 of the NBRF database.

Example 4: Whole Mount in situ, Enhancer Trap Detection and Antibody Labeling

Whole mount in situ hybridizations were conducted using digoxigenin-derivatized DNA probes from cDNA B52-5. Immunocytochemistry was done essentially as described in Rothberg et al, 1988, supra. Anti-β-galactosidase antibody (Promega Corp.) was used to detect the signal from the enhancer trap constructs and detected with a horseradish peroxidase (HRP)-conjugated anti-mouse antibody (Jackson Immunological Laboratories). Signal from whole mount in situ is cytoplasmic (Tautz, D., and Pfeiffle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals transnational
control of the segmentation gene hunchback. Chromosoma 98, 81-85) enhancer trap signal is localized to the nucleus (Bellen et al., 1990), and antibody staining shows both cytoplasmic and cell surface staining.

Example 5: **Immunoelectron and Confocal Microscopy**

All preparations were made by dissecting embryos in Schneider medium to expose the nerve cord. Samples were fixed in 2% paraformaldehyde with .025% glutaraldehyde for 15 minutes followed by primary and secondary antibody labeling without detergent. Primary E.M. fixation was performed using 2% glutaraldehyde and 2% paraformaldehyde prior to silver enhancement of signal from the HRP-conjugated secondary (Amersham Corp). The silver enhancement procedure prevents accurate distinctions to be made concerning the relative levels of antigen present among subsets of axons. Samples were treated with 1% OsO₄ and counter-stained with Uranyl-acetate. Sections were prepared on a Reichert ultramicrotome and visualized on a Jeol electron microscope. Confocal images were made using a Biorad MRC 500 system and a Zeiss Axiovert compound microscope.

Example 6: **Immunofluorescence, Immunoprecipitations, and Immunoblots**

Immunofluorescence of Drosophila S2 cell lines, the preparation of lysates from Canton-S embryos and S2 cell lines (Schneider, I. (1972). Cell lines derived from late embryonic stages of Drosophila melanogaster. J. Embryol. exp. Morph 27, 353-365) were performed essentially as described in Fehon, R. G., Kooh, P. J., Rebay, I., Regan C. L., Xu, T., Muskavitch, M. A. T., and Artavanis-Tsakonas, S. (1990). Molecular interactions between the protein products of the neurogenic loci Notch and Deta, two EGF-homologous genes in Drosophila. Cell 61, 523-534. Immunoprecipitation of protein lysates and S2 cell conditioned media were performed with anti-slit
antibodies followed by the precipitation of the immune complex with protein A-sepharose 6MD (Pharmacia) or protein A/G beads (Pierce). Samples were suspended in SDS-PAGE loading buffer, boiled, and separated by SDS-PAGE. Following transfer to nitrocellulose, blots were probed with anti-slit antibodies and detected with HRP-conjugated goat anti-rabbit antibodies. No immunoprecipitable species from KC cell lysates or conditioned media was detected by immunoblotting matrix proteins deposited by S2 cells grown in plastic culture flasks (T75; Corning) were prepared, after removal of the cells and 3 rinses with 1X PBS, by directly boiling in 300-500 µl of SDS-PAGE loading buffer. 5-10µl were used per lane for immunoblot analysis. Detection of S35 labeled slit protein in the media was performed by metabolically labelling (0.1mCi/ml, ICN translabel) S2 cells for 4 hours in M3 media (minus methionine and cysteine), followed by immunoprecipitating the conditioned media with anti-SLIT antibody and protein A-sepharose 6MD. Precipitates were washed overnight in PBS with 1% bovine serum albumin and 0.1% TRITON followed by separation with SDS-PAGE and autoradiography.

Example 7: Purification of Pure and Active SLIT protein

Conditioned media from tissue culture cells expressing the natural form of the SLIT protein or detergent extracts of protein lysates expressing SLIT are passed through an antibody column consisting of anti-SLIT IgG monoclonal antibody coupled to Sepharose CL beads (10 mg Mab/ml swollen beads). The column is then washed with 10 bed volumes of PBS and 0.1% TRITON. The protein is then eluted using a 50 mM diethylamine-HCl pH 11.5 and 0.5% deoxycholate buffer and neutralized with glycine. The eluted fractions are monitoring by antigenic activity and shown to be in pure form by SDS-PAGE. The biological activity of the protein is monitored by an axonal outgrowth assay. The same procedure is used to isolate and assay recombinant forms of the SLIT protein.
consisting of the various sequence elements defined in this application. Stable Drosophila cell lines over expressing the SLIT protein were constructed by cloning the coding portions of the SLIT gene into the metallothionein promoter vector pRMHa-3 (Bunch et al, 1988 et al., Characterization and use of the Drosophila metallothionein promoter in cultured Drosophila melanogaster cells. Nucl. Acids Res. 16, 1043-1061) and transfecing into the S2 cell lines (Schneider, 1972).

It will be appreciated that the instant specification is set forth by way of illustration and not limitation, and that various modifications and changes may be made without departing from the spirit and scope of the present invention.
SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) Applicant: Rothberg, Jonathan Marc and Artavanis-Tsakonas, Spyridon
(ii) TITLE OF INVENTION: Purified SLIT protein and Sequence Elements
Thereof
(iii) NUMBER OF SEQUENCES: 9
(iv) CORRESPONDENCE ADDRESS:
    (A) ADDRESSEE: Yale University
        Office of Cooperative Research
    (B) STREET: 246 Church Street
        Suite 401
    (C) CITY: New Haven
    (D) STATE: Connecticut
    (E) COUNTRY: USA
    (F) ZIP: 06510

(v) COMPUTER READABLE FORM:
    (A) MEDIUM TYPE: Diskette, 3.50 inch. 800 Kb storage
    (B) COMPUTER: Apple Macintosh
    (C) OPERATING SYSTEM: Macintosh 6.0.5
    (D) SOFTWARE: Microsoft Word 4.0

(vi) CURRENT APPLICATION DATA:
    (A) APPLICATION NUMBER: 07/624,135
    (B) FILING DATE: 7-DEC-1990
    (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA: not applicable

(viii) ATTORNEY INFORMATION:
    (A) NAME: Barth, Richard J.
    (B) REGISTRATION NUMBER: 28,180
    (C) REFERENCE/DOCKET NUMBER: 900964/RSB

(ix) TELECOMMUNICATION INFORMATION:
    (A) TELEPHONE: (212) 972-1400
    (B) TELEFAX: (212) 370-1622
    (C) TELEX: 236268
(2) INFORMATION FOR SEQ ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8378
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) ORIGINAL SOURCE:
(A) ORGANISM: Drosophila Melanogaster
(D) DEVELOPMENTAL STAGE: embryonic and larval, germ-line derived.

(vi) IMMEDIATE SOURCE:
(A) LIBRARY: cDNA and Genomic
(B) CLONE: be2.4, ka2.4, B52-2, B52-5, smart2-19

(vii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: 2R
(B) MAP POSITION: 52D
(C) UNITS: chromosome band

(ix) FEATURE:
(A) NAME/KEY: 5' leader sequence
(B) LOCATION: 1 to 314
(C) IDENTIFICATION METHOD: experimental

(A) NAME/KEY: Translated region
(B) LOCATION: 315 to 4754
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: codes for slit protein

(A) NAME/KEY: 3' untranslated region
(B) LOCATION: 4755 to 8378
(C) IDENTIFICATION METHOD: experimental

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

TCAGTTTGGT CAGAAGCCG CTTGGCGGACG GCTGCAAAAG AGCGTACC GC CTTGGAAAA 60
CCCGAGAGA AAAGTGCCGC GTGGAGCGGG GCGGACATTCC ACCGAAACCA AAAGCCTCG 120
AAGCTGATAT CGAATCAGAA GGATTAATCC AGTGAATCA GTGAAGTGAA AGTGCCCTGG 180
AAGGCATCAT CAATCTTTTA TCTTTTCTCC CTCAATAATT TACCAGTGG TGATTTGCTT 240
TGACAAAGTG GATGGCCATA TACGGGGGCC ACTTTCAATT AGGCCAGTTG CGCCTGCTTC 300
ATAAAATGTC CACA 314

ATG GCC GCG CCG TCC AGG ACC ACG AGC TTG ATG CCA CCA CCA TTC CCG 359
Met Ala Ala Pro Ser Arg Thr Thr Leu Met Pro Pro Pro Pro Arg

20 10 15

CTC CAG CTG CCG CTA CTG ATA CTA CCC ATC CTG CTA CTC CTG CGC 404
Leu Gln Leu Arg Leu Leu Ile Leu Leu Pro Ile Leu Leu Leu Leu

25 20 30
CAT GAT GCG GTC CAC GCG GAA CCG TAT TCC GCC GGA TTC GCC AGC 449
  His Asp Ala Val His Ala Glu Pro Tyr Ser Gly Gly Phe Gly Ser
  35
  40
  45
TCA GCT GTA TCC AGC GGT GGA CTG GGG TCA GTG GCC ATT CAC ATA 494
  Ser Ala Val Ser Gly Gly Leu Gly Ser Val Gly Ile His Ile
  50
  55
  60
CCC GGC GGC GGA GTG GGC GTC ATC ACG GAG GCC GGC TGC CCG AGG 539
  Pro Gly Gly Val Gly Val Ile Thr Glu Ala Arg Cys Pro Arg
  65
  70
  75
GTC TGC TCC TGC ACC GCA TTA AAT GTG GAT TGC TCC CAT CGA GGA 584
  Val Cys Ser Cys Thr Gly Leu Val Asn Val Asp Cys Ser His Arg Gly
  80
  85
  90
CTC ACC TCC GTT CCC AGG AAA ATC TCA GCG GAC GTG GAG CGA CTC 629
  Leu Thr Ser Val Pro Arg Lys Ile Ser Ala Asp Val Glu Arg Leu
  95
  100
  105
GAG CTG CAG GGA AAC AAT TTG ACC GTC ATA TAC GAG ACG GAT TGC 674
  Glu Leu Gln Gly Asn Asn Leu Thr Val Ile Tyr Glu Thr Asp Phe
  110
  115
  120
CAG CGG CTG ACC AAG CTG CGA ATC CTC CAA CTA ACT GAC AAT CAG 719
  Gln Arg Leu Thr Lys Leu Arg Met Leu Gln Leu Thr Asp Asn Gln
  125
  130
  135
ATC CAC ACG ATC GAG AGG AAC TCC TCC CAA GAT TTG GTC TCA CTC 764
  Ile His Thr Ile Glu Arg Asn Ser Phe Gln Asp Leu Val Ser Leu
  140
  145
  150
GAG CGA CTG GAC ATC TCC AAC AAT GTC ATC ACG ACC GTG GGT AGA 809
  Glu Arg Leu Asp Ile Ser Asn Asn Val Ile Thr Val Gly Arg
  155
  160
  165
GCC GTC TTC AAG GGA GCC CAA TCG TTG CGG AGT CTT CAG CTC GAC 854
  Arg Val Phe Lys Gly Ala Gln Ser Leu Arg Ser Leu Gln Leu Asp
  170
  175
  180
AAT AAC CAA ATC ACC TGC CTG GAT GAG CAC GCC TTT AAG GGA TTG 899
  Asn Asn Gln Ile Thr Cys Leu Asp Glu His Ala Phe Gly Leu
  185
  190
  195
GTG GAG CTG GAG ATA CTC ACG CTG AAC AAG AAC AAG CTG ACT TCC 944
  Val Glu Leu Glu Ile Thr Leu Asn Asn Asn Leu Thr Ser
  200
  205
  210
CTG CGG CAC ATC TTC GCC GGA CTG GGA GTG TTG CGG GCA CTC 989
  Leu Pro His Asn Ile Phe Gly Gly Leu Gly Arg Leu Arg Ala Leu
  215
  220
  225
CGG CTG TCG GAC AAT CGG TTC GCC TGC GAC TGC CAT CTG TCC TGG 1034
  Arg Leu Ser Asp Asn Pro Phe Ala Cys Asp Cys His Leu Ser Trp
  230
  235
  240
CTG TCG CGA TTC CTT CGC AGT GCC ACC CGC CTG GCG CCC TAC ACC
Leu Ser Arg Phe Leu Arg Ser Ala Thr Arg Leu Ala Pro Tyr Thr
  245     250     255

GCC TGC CAG TCG CCA TCG CAG CTG AAG GGC CAA AAC GTC GCG GAC
Arg Cys Gln Ser Pro Ser Gln Leu Lys Gly Gln Asn Val Ala Asp
  260     265     270

CTG CAC GAC CAG GAG TTC AAA TGC TCG GGT CTG AGC GAG CAC GQA
Leu His Asp Gln Phe Lys Cys Ser Gly Leu Thr Glu His Ala
  275     280     285

CCG ATG GAA TGC GGG GCG GAG AAC AGC TGT CGG CAC CCA TGT CGC
Pro Met Glu Cys Gly Ala Glu Asn Ser Cys Pro His Pro Cys Arg
  290     295     300

TGT GCG GAC GGG ATC GTC GAT TGC GCT GAG AAG AGT CTG ACC AGC
Cys Ala Asp Gly Ile Val Asp Cys Arg Glu Lys Ser Leu Thr Ser
  305     310     315

GTG CCC GTC ACC TTG CCC GAC GAC ACC ACC GAC GTT CGC CTC GAG
Val Pro Val Thr Leu Pro Asp Thr Thr Asp Val Arg Leu Glu
  320     325     330

CAA AAT TTC ATT ACG GAA CTG CGC CGG AAA TCG TTC TCC AGC TTT
Gln Asn Phe Ile Thr Thr Arg Cys Leu Pro Lys Ser Phe Ser Ser Phe
  335     340     345

CGA CGA CTG CGA CGC ATC GAC CTG TCC AAC AAC AAT TCC CGG
Arg Arg Leu Arg Arg Ile Asp Leu Ser Asn Asn Ile Ser Arg
  350     355     360

ATT GCC CAC GAT GCA CTA AGC GCC CTA AAG CAG TTA ACC ACT CTC
Ile Ala His Asp Ala Leu Ser Gly Leu Lys Gln Leu Thr Thr Leu
  365     370     375

GTG CTG TAC GGC AAT AAA ATA AAG GAT TTA CCC TCG GGC GTG TCC
Val Leu Tyr Gly Asn Lys Ile Lys Asp Leu Pro Ser Gly Val Phe
  380     385     390

AAA GGA CTC GCC TGC CTC AGG CTG CTG CTG AAC GCC AAC GAG
Lys Gly Leu Gly Ser Leu Arg Leu Leu Leu Asn Ala Asp
  395     400     405

ATC TCG TGC ATA CGC AAG GAT GCC TTT CGC GAC CTG CAC AGT TTG
Ile Ser Cys Ile Arg Lys Ala Phe Arg Asp Leu His Ser Leu
  410     415     420

AGC CTG CTC TCC TGT AAC AAG ATC CAG TCG CTG GCT AAT
Ser Leu Leu Ser Leu Tyr Asp Asn Ile Gln Ser Leu Ala Asn
  425     430     435

GGC ACA TTC GAC GCC ATG AAG AGC ATG AAA ACG GTA CAT CTG GCC
Gly Thr Phe Asp Ala Met Lys Ser Met Lys Thr Val His Leu Ala
  440     445     450
AAG AAT CCT TTC ATC TGC GAC TGC AAT CTG CGC TGG CTG GCC GAC 1709
 Lys Asn Pro Phe Ile Cys Asp Asp Asn Leu Arg Trp Leu Ala Asp
 455       460       465
TAT TTG CAC AAA AAT CCC ATA GAG ACG AGT GGA GCC CGC TGC GAG 1754
 Tyr Leu His Lys Asn Pro Ile Glu Thr Ser Gly Ala Arg Cys Glu
 470       475       480
TCA CCG AAG CGG ATG CAT CCT CGT CGG ATT GAA TCG CTG CGC GAG 1799
 Ser Pro Lys Arg Met His Arg Arg Arg Ile Glu Ser Leu Arg Glu
 485       490       495
GAG AAA TTC AAA TGC TCC TGG GCT GAA TTG CGG ATG AAG CTG TCG 1844
 Glu Lys Phe Lys Cys Ser Trp Gly Glu Leu Arg Met Lys Leu Ser
 500       505       510
GCC GAG TGC CGG ATG GAC TCC GAG TTG CGC ATG TGC CAC TGC 1889
 Gly Glu Cys Arg Met Asp Ser Asp Cys Pro Ala Met Cys His Cys
 515       520       525
GAG GCC ACC ACC GTG GAT TGC ACG GCC CGG CGG CTG AAG GAG ATT 1934
 Glu Gly Thr Thr Val Asp Cys Thr Gly Arg Arg Leu Lys Glu Ile
 530       535       540
CCG CGC GAC ATT CCC CTG CAC ACA ACT GAG CTT TTG CTC AAC GAC 1979
 Pro Arg Asp Ile Pro Leu His Thr Thr Glu Leu Leu Leu Asn Asp
 545       550       555
AAC GAA CTG GGA CGC ATC AGT TCC GAT GCC CTC TTT GGT CGC CTG 2024
 Asn Glu Leu Gly Arg Ile Ser Ser Asp Gly Leu Phe Gly Arg Leu
 560       565       570
CCG CAC TTG GTG AAG CTG GAA TTG AAG CGC AAC CAG CTG ACC GCC 2069
 Pro His Leu Val Lys Leu Glu Leu Lys Arg Asn Gln Leu Thr Gly
 575       580       585
ATC GAG CCC AAC GCC TTC GAG GGA GCA TCC CAC ATC CAG GAG TTG 2114
 Ile Glu Pro Asn Ala Phe Glu Gly Ala Ser His Ile Gln Glu Leu
 590       595       600
CAG CTG GGC GAG AAC AAG ATC AAG GAG ATA TCG AAC AAG ATG TTC 2159
 Gln Leu Gly Glu Asn Lys Ile Lys Glu Ile Ser Asn Lys Met Phe
 605       610       615
CTG GGA CTG CAC CAA CTA AAA ACG CTC AAT CTG TAC GAC AAT CAA 2204
 Leu Gly Leu His Gln Leu Lys Thr Leu Asn Leu Tyr Asp Asn Gln
 620       625       630
ATC TCA TGC GTT ATG CCC GGT TTG GAG CAT CTC AAC TCT GTC 2249
 Ile Ser Cys Val Met Pro Gly Ser Phe Glu His Leu Asn Ser Leu
 635       640       645
ACG TCG CTG AAC CTC GCA TCG AAT CCA TTC AAT TGC AAT TGT CAT 2294
 Thr Ser Leu Asn Leu Ala Ser Asn Pro Phe Asn Cys Asn His
 650       655       660
<table>
<thead>
<tr>
<th>Sequence</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTG GCC TGG TTC GCG GAA TGC GTG CGC AAA AAA TCA CTG AAC GGC</td>
<td>2339</td>
</tr>
<tr>
<td>Leu Ala Trp Phe Ala Glu Cys Val Arg Lys Lys Ser Leu Asn Gly</td>
<td>655</td>
</tr>
<tr>
<td></td>
<td>670</td>
</tr>
<tr>
<td></td>
<td>675</td>
</tr>
<tr>
<td>GGA GCG GCA CTT TGT GGA GCC CCG TCG AAG GTA CTG GAC GTG CAG</td>
<td>2384</td>
</tr>
<tr>
<td>Gly Ala Ala Arg Cys Gly Ala Pro Ser Lys Val Arg Asp Val Gln</td>
<td>680</td>
</tr>
<tr>
<td></td>
<td>685</td>
</tr>
<tr>
<td></td>
<td>690</td>
</tr>
<tr>
<td>ATC AAG GAC TGG CCC CAC TCG GAA TTC AAG TGT AGC AGC GAG AAC</td>
<td>2429</td>
</tr>
<tr>
<td>Ile Lys Asp Leu Pro His Ser Glu Phe Lys Cys Ser Ser Glu Asn</td>
<td>695</td>
</tr>
<tr>
<td></td>
<td>700</td>
</tr>
<tr>
<td></td>
<td>705</td>
</tr>
<tr>
<td>AGC GAG GGC TGC CTG GCC GAT GCC TAC TGT CCG CCA TCC TGC ACC</td>
<td>2474</td>
</tr>
<tr>
<td>Ser Glu Gly Cys Leu Gly Asp Gly Tyr Cys Pro Pro Ser Cys Thr</td>
<td>710</td>
</tr>
<tr>
<td></td>
<td>715</td>
</tr>
<tr>
<td></td>
<td>720</td>
</tr>
<tr>
<td>TGC ACC GCC ACC GTG GTC GCC TGG TCG GTG AAG CAG CTG AAG GAG</td>
<td>2519</td>
</tr>
<tr>
<td>Cys Thr Gly Thr Val Val Ala Cys Ser Arg Asn Gln Leu Lys Glu</td>
<td>725</td>
</tr>
<tr>
<td></td>
<td>730</td>
</tr>
<tr>
<td></td>
<td>735</td>
</tr>
<tr>
<td>ATA CGG CGA ATT CCC GCC GAA ACA TGG GAG CTG TAT CTG GAG</td>
<td>2564</td>
</tr>
<tr>
<td>Ile Pro Arg Gly Ile Pro Ala Glu Thr Ser Glu Leu Tyr Leu Glu</td>
<td>740</td>
</tr>
<tr>
<td></td>
<td>745</td>
</tr>
<tr>
<td></td>
<td>750</td>
</tr>
<tr>
<td>TCC AAT GAG ATC GAG CAG ATT CAC TAC GAA GCC ATA GCC CAT TTG</td>
<td>2609</td>
</tr>
<tr>
<td>Ser Asn Glu Ile Glu Gln Ile His Tyr Glu Arg Ile Arg His Leu</td>
<td>755</td>
</tr>
<tr>
<td></td>
<td>760</td>
</tr>
<tr>
<td></td>
<td>765</td>
</tr>
<tr>
<td>CGC TCC CTT ACC CGA CTC GAT TCT ACC AGC AAC AAG CAG ATC ACC ATT</td>
<td>2654</td>
</tr>
<tr>
<td>Arg Ser Leu Thr Arg Leu Asp Leu Ser Asn Asn Gln Ile Thr Ile</td>
<td>770</td>
</tr>
<tr>
<td></td>
<td>775</td>
</tr>
<tr>
<td></td>
<td>780</td>
</tr>
<tr>
<td>CTT TGC ACC TAC ACC TTT GCC AAT CTG ACC AAG CTG TCC ACG CTC</td>
<td>2699</td>
</tr>
<tr>
<td>Leu Ser Asn Tyr Thr Phe Ala Asn Leu Thr Lys Leu Ser Thr Leu</td>
<td>785</td>
</tr>
<tr>
<td></td>
<td>790</td>
</tr>
<tr>
<td></td>
<td>795</td>
</tr>
<tr>
<td>ATC ATC TCA TAC AAG CTG CAG TCT GTG CAG CGG CAT GCC TTG</td>
<td>2744</td>
</tr>
<tr>
<td>Ile Ile Ser Tyr Asn Leu Glu Cys Leu Glu Arg His Ala Leu</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>805</td>
</tr>
<tr>
<td></td>
<td>810</td>
</tr>
<tr>
<td>TCT GCC CTG AAT ACC CTG GCC GTC GTT TCG CAC GGT AAC GGC</td>
<td>2789</td>
</tr>
<tr>
<td>Ser Gly Leu Asn Asn Leu Arg Val Val Ser Leu His Gly Asn Arg</td>
<td>815</td>
</tr>
<tr>
<td></td>
<td>820</td>
</tr>
<tr>
<td></td>
<td>825</td>
</tr>
<tr>
<td>ATC TCG ATG CTG GCC GAA GCC TCC TTC TCG GAG CTC AAC TCG TTG</td>
<td>2834</td>
</tr>
<tr>
<td>Ile Ser Met Leu Pro Glu Gly Ser Phe Glu Asp Leu Lys Ser Leu</td>
<td>830</td>
</tr>
<tr>
<td></td>
<td>835</td>
</tr>
<tr>
<td></td>
<td>840</td>
</tr>
<tr>
<td>ACC CAC ATC GCA CTA GCC AGC AAT CCC TTG TAC TGC GAC TCG GGT</td>
<td>2879</td>
</tr>
<tr>
<td>Thr His Ile Ala Leu Gly Ser Asn Pro Leu Tyr Cys Asp Cys Gly</td>
<td>845</td>
</tr>
<tr>
<td></td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>855</td>
</tr>
<tr>
<td>CTA AAG TGG TTC TCC GAT TGG ATC AAG CTG GAC TAC GTG GAA CCG</td>
<td>2924</td>
</tr>
<tr>
<td>Leu Lys Trp Phe Ser Asp Trp Ile Lys Leu Asp Tyr Val Glu Pro</td>
<td>860</td>
</tr>
<tr>
<td></td>
<td>865</td>
</tr>
<tr>
<td></td>
<td>870</td>
</tr>
</tbody>
</table>
GGA ATT GCA CTT TGC GCC GAA CCG GAA CAG ATG AAG GAT AAG CTG
Gly Ile Ala Arg Cys Ala Glu Pro Glu Gln Met Lys Asp Lys Leu
875  880  885

ATC CTG TCC ACA CCC TCG TGC AGC TTC TGC CCG GCC GGC CTG
Ile Leu Ser Thr Pro Ser Ser Ser Phe Val Cys Arg Gly Arg Val
890  895  900

CGC AAT GAT ATT CCG GCC AAG TGC AAC GCC TGT TTC GAG CAG CCA
Arg Asn Asp Ile Leu Ala Lys Cys Asn Ala Cys Phe Glu Gln Pro
905  910  915

TGC CAG AAT CAG GCG CAG TGT GTG GCC CTT CCG CAG CGA GAG TAC
Cys Gln Asn Gln Ala Gln Cys Val Ala Leu Pro Gln Arg Glu Tyr
920  925  930

CAG TGC CTC TGC CAG CCG GCC TAT CAT GCG AAA CAC TGT GAG TTT
Gln Cys Leu Cys Gln Pro Gly Tyr His Gly Lys His Cys Glu Phe
935  940  945

ATG ATC GAT GCT TGC TAC GGA AAT CCG TGC CGC AAC AAT GCC ACC
Met Ile Asp Ala Cys Tyr Gly Asn Pro Cys Arg Asn Ala Thr
950  955  960

TGC ACG GTG CTG GAG GAT GGT CAG TCG TAC GAT CTT AGC TGG TAC
Cys Thr Val Leu Glu Glu Gly Arg Phe Ser Cys Gln Cys Ala Pro
965  970  975

GGA TAC ACA GGT GCC CGC TGC GAG ACG AAT ATC GAC GAT TGC CTG
Gly Thr Thr Gly Ala Arg Cys Glu Thr Asn Ile Asp Asp Cys Leu
980  985  990

GAG ATC AAG TGC CAG AAC AAT GCC ACC TGC ATC GAC GGA GTG
Gly Glu Ile Lys Cys Gln Asn Asn Ala Thr Cys Ile Asp Gly Val
995 1000 1005

GAG TGC TAC AAA TGT GAG TGC CAG CCG GGA TTC AGT GGC GAG TTT
Glu Ser Tyr Lys Cys Glu Cys Glu Pro Gly Phe Ser Gly Glu Phe
1010 1015 1020

TGC GAC ACC AAA ATC CAG TCC TGC AGT CCG GAG TTC AAT CCC TGC
Cys Asp Thr Lys Ile Glu Phe Cys Ser Pro Glu Phe Asn Pro Cys
1025 1030 1035

GCG AAT GGA GCC AAG TGC ATG GAC CAC TTT ACC CAC TAC AGC TGG
Ala Asn Gly Ala Lys Cys Met Asp His Phe Thr His Tyr Ser Cys
1040 1045 1050

GAT TGT CAG GCA GGT TTC CAT GGC ACC AAC TGC ACG GAC AAT ATT
Asp Cys Glu Ala Gly Phe His Gly Thr Asn Cys Thr Asp Asn Ile
1055 1060 1065

GAC GAC TGC CAG AAC CAC ATG TGC CAG AAC GGT GGA ACG TGC GTG
Asp Asp Cys Glu Asn His Met Cys Glu Asn Gly Thr Cys Val
1070 1075 1080
GAC GGC ATC AAC GAC TAC CAA TGC CGC TGT CCA GAC GAC TAT ACG 3599
Asp Gly Ile Asn Asp Tyr Gln Cys Arg Cys Pro Asp Asp Tyr Thr 1085 1090 1095
GGC AAG TAC TGT GAA GCC CAC AAC ATG ATC TCG ATG ATG TAT CCA 3644
Gly Lys Tyr Cys Glu Gly His Asn Met Ile Ser Met Met Tyr Pro 1100 1105 1110
CAG ACG TCG CCT TGT CAA AAC CAC GAG TGC AAG GAC GGT GTC TGC 3689
Gln Thr Ser Pro Cys Gln Asn His Glu Cys Lys His Gly Val Cys 1115 1120 1125
TTC CAA CCG AAC GCT CAG GGC AGC GAC TAC CTA TCG AGG TGT CAT 3734
Phe Gln Pro Asn Ala Gln Gly Ser Asp Tyr Leu Cys Arg Cys His 1130 1135 1140
CCG GGT TAC ACT GGA AAG TGG TCG GAG TAC TCT ACC AGC ATT AGC 3779
Pro Gly Tyr Thr Gly Lys Trp Cys Glu Tyr Leu Thr Ser Ile Ser 1145 1150 1155
TTC GTC CAC AAC TCG TTT GTG GAA CTG GAG CCA CTG CGA ACA 3824
Phe Val His Asn Ser Phe Val Glu Leu Glu Pro Leu Arg Thr 1160 1165 1170
CGT CCG GAG GCC AAC GTG ACG ATA GTC TTC AGC AGC GCG GAG CAG 3869
Arg Pro Glu Ala Asn Val Thr Ile Val Phe Ser Ser Ala Glu Gln 1175 1180 1185
AAT GGA ATT CTC ATG TAG GAC GCC CAG GAT GCA CAT CTC GCA GTG 3914
Asn Gly Ile Leu Met Tyr Asp Gly Gln Asp Ala His Leu Ala Val 1190 1195 1200
GAG CTG TTT AAT GGG CGT ATT CGG GTT AGC TAC GAT GTG GGT AAT 3959
Glu Leu Phe Asn Gly Arg Ile Arg Val Ser Tyr Asp Val Gly Asn 1205 1210 1215
CAC CCT GTG TCC AGC ATG TAC AGC TTT GAA ATG GTG GCC GAT GGA 4004
His Pro Val Ser Thr Met Tyr Ser Phe Glu Met Val Ala Asp Gly 1220 1225 1230
AAG TAC CAT GCC GTG GAG CTT CTG GCC ATC AAG AAG AAT TCC ACG 4049
Lys Tyr His Ala Val Glu Leu Leu Ala Ile Lys Asn Phe Thr 1235 1240 1245
CTG CGC GTG GAT CGG TGA TG GCC CGT TCC ATC ATC AAG GAG GCC 4094
Leu Arg Val Asp Arg Gly Leu Ala Arg Ser Ile Ile Asn Gly Gln 1250 1255 1260
TCC AAC GAC TAC CTG AAA CCT AGC ACT CGG ATG TCC GTG GCC GCC 4139
Ser Asn Asp Tyr Leu Lys Leu Thr Thr Pro Met Phe Leu Gly Gly 1265 1270 1275
CTA CCA GTG GAT CCT GCA CAG CAG CAC TAC AAG TGG CAA ATA 4184
Leu Pro Val Asp Pro Ala Gln Gln Ala Tyr Lys Asn Trp Gln Ile 1280 1285 1290
-81-

CGC AAC CTT ACC AGC TTT AAG GCC TGC ATG AAG GAG GTG TGG ATC 4229
Arg Asn Leu Thr Ser Phe Lys Gly Cys Met Lys Glu Val Trp Ile
1295 1300 1305

AAT CAT AAG CTT GTC GAC TTT GCC AAT GCC CAG CGC CAG CAA AAG 4274
Asn His Lys Leu Val Asp Phe Gly Asn Ala Gln Arg Gln Gln Lys
1310 1315 1320

ATC ACA CCA GGA TGT GCC CTG CTC GAA GGA GAG CAG CAA GAG GAG 4319
Ile Thr Pro Gly Cys Ala Leu Leu Gly Glu Glu Gln Glu Glu
1325 1330 1335

GAA GAC GAC GAG CAG GAT TTC ATG GAC GAG ACA CCG CAC ATC AAA 4364
Glu Asp Asp Glu Gln Asp Phe Met Asp Glu Thr Pro His Ile Lys
1340 1345 1350

GAG GAG CCG GTG CAT CCT TGC GTG GAG AAC AAA TGC CGT CGG GGC 4409
Glu Glu Pro Val Asp Pro Cys Leu Glu Asn Lys Cys Arg Arg Gly
1355 1360 1365

AGT CGC TGT GTG CGG AAT TCC AAT GCC AGG GAC GCC TAC GAG TGC 4454
Ser Arg Cys Val Pro Asn Ser Asn Ala Arg Asp Gly Tyr Gln Cys
1370 1375 1380

AAG TGC AAG CAC GCC CAG CGC GCC TAC TGC GAT CAA GGT GAG 4499
Lys Cys Lys His Gly Glu Arg Gly Arg Tyr Cys Glu Gln Gly Glu
1385 1390 1395

GCG AGC ACT GAG CCC CCA ACA GTC ACC CCG GCG TCC ACC TGC GCC 4544
Gly Ser Thr Glu Pro Pro Thr Val Thr Ala Ala Ser Thr Cys Arg
1400 1405 1410

AAG GAG CAG GTG CGC GAG TAC TAC ACG GAG AAC GAC TGT CGC TGG 4589
Lys Glu Gln Val Arg Glu Tyr Tyr Thr Glu Asp Asp Cys Arg Ser
1415 1420 1425

AGG CAG CCG TTG AAG TAC GCC AAG TGC GTG GCC GGC TGC GGC AAC 4634
Arg Glu Pro Leu Lys Tyr Ala Lys Cys Val Gly Gly Cys Gly Asn
1430 1435 1440

CAG TGC TGC GCG GCC AAA ATT GTG AGA CGG CGC AAG GTG CGC ATG 4679
Gln Cys Cys Ala Leu Lys Ile Val Arg Arg Arg Lys Val Arg Met
1445 1450 1455

GTG TGC AGC AAC AAG CGC AGG TAC ATC AAG AAC TTG GAC ATC GTG 4724
Val Cys Ser Asn Asn Arg Lys Tyr Ile Lys Asn Leu Asp Ile Val
1460 1465 1470

CGC AAG TGC GGA TGC ACC AAG AAA TGC TAC 4754
Arg Lys Cys Gly Cys Thr Lys Lys Cys Tyr
1475 1480

TGACTG AAAGATGGCA CTACCCAATT GCTCGAACGG AGCAAATAGCA 4800

GCCTTAGAGT TACGTTTAA ACAGGTTTAA ATCTAACTTA TAGTAGTAGT AATAGTAACG 4860

ATAGCTTAG CCATAGCACT AGGATAGCA CGATGTTTAG GGGACGAAG GATGAAGTGG 4920
AGGAGAGTGCC TGACCGGGGG GAGCAACCGG AGCGAGTGAG GAGACAGAGT GATGATTACC 4980
CCACCGACGG CATCGCAGTT GATCTTACAG AGCAGAACC AGATGACAGG GATGACGATG 5040
GTCTCGATGA TGATATAGCA GACAGGAGG AATGGGAGGA GATCCAGAG CAGCTTCCCG 5100
ATCCCAAGGG TCTCGTAGGT CGTCCAGATG AAGAGAAAGA CATGGTTAAC GACAGGATG 5160
ACGAACCTAT GCGCATGGAG GGGCGAAGAA GGTTTYAGGAC CAGCTGCGGC GAGGACATT 5220
TCCTTACCGA AGAGGGCGAG GTGTTGGGTTG CTTTCTGATC GCGATTCGCG CCGACATTG 5280
GCTCCGGCCA GACTCAGCTG GAGAATATCC GCAAGAAGCT GTCACAGAAA GCACAGCCGG 5340
CTCCTGGGAAAC GCTGTTGGCG GTGCGCCGTC CGAGCATGAC GATAGATCTG GCGGAGACCA 5400
CCGGCAGATTT TGCACCGGAT GAGAGAGGCG GCGGGCTTTT CTTGCTCAGG CACAAACACG 5460
AATTCCGGGA CAGGGGCGAG AACACGGGCG CGCGGCTTTTG TGGCTCGGAC CAGCAACACG 5520
GCAAGAACGG TCCGTATCAG CGCAAGACGC GCAACGATAG CATCAAATAC ATCTCCAGGG 5580
CGCTGGCCAA GGTGAGACCT GTGTACGACG AGACGGACAA GGACAAAGTG CGGACAAGGG 5640
ATGCCCAAACA GACGCAGCAA AANAAAGCAG CGCTCAGCAG CTTGCGACGC CTGTCAAGGG 5700
ACCCCGAGAG CAGTCTAGCC TTTCGGGTCC CCGACCCCAA GATACACCTT GTTCTGACGG 5760
CCGATGGCAA GGTGGCGCTG CTCTATCGGC GAGACTCGGA GAGTCAAGG TAGGAGGCA 5820
TAGCGACCTT GACGCAACAG TTCTCGGGAC AGCCCGCCAA GAGTGCAAGA CCTAAAACCG 5880
AAATTCTTCT CTGCCGCGAG GACTCTNTCT ACACGGACAG CGAGGATACC GAGGACAGTA 5940
AAGGTAAAC TACGTGCTGA AAGTCCCAAC CAGTTGCGCG CACGCCCCAC CACTCAGGAC 6000
CAGAGATTTT GCCCGTCCTA GATAACGTCC AGCGAGGAGA GTTATTTATA ATTCGGCCCA 6060
CCTCGAGACT CCTTCTGCGG ATGATCAACA GGGCAGTGTG CGAGGTTGTC GGCAATCAAGA 6120
AGAACCGATT CGCGGAGAAC CGGTGGGCGT ACCAATGCCC CAGGACAGAC CCTCGCGGCC 6180
CAGTCCCGGA GCGCAGGTCC GCTAGTCGGC CTTCCGGGACA TCAGTTTCTA GCCAAAGTTG 6240
ACCTGGCTGA GTCCCGAGAC TCCGGAGGCA CGCTCGAGAC CGCGCTGATA CCCAGACCC 6300
ACGACTTCTG CTTCCAGCGT GACAACAGCA TGCTGGAGGA GCGTGCGGCG GTGCTGAGT 6360
TGGAAAAGCA GCCGGAGCGG GACAAAGGAG ACAACAGGCC CACCAGCAAG GGAGGACCG 6420
AGGCACACAC CATAGCATA CGAGGACGCT CTCTCCAGAG CGAAAGCTCA CAACTCGGAC 6480
ATGCTGCCAC AGTTTGGAGA GACACTTGGAG GCTTACAGGC GCTGCGAGGA GAAATGGTGT 6540
CGGGCGCCAC ATAGGGGGCG ACATCGTCAT CGGGACAAGC CGGGAGGAGA ACTGTGCTCC 6600
ATCCATTGCA TCATGCAGGT CATGATGGCG TGGCCGGCGT GTCGACGTCG TTTGCGCATG 6660
TG GCCACCTT TTTCAAGCAA CGCATCCTCG ATCATGTCCG GGTAGTCAA 5720
CAAGGGGAT TCCATTTTG TGTCGAATG CCCACATTC CCCACCCCA ACCCCGTTTC 6780
CTGCAGTCTC ATGATCATC TGAGTCTCT GAGGCTGCG TAGGTGCCTT CGGATGCGA 6840
GGTCCCTCTT CTACTACTAG CACTATATA TCAGAATATA TACTGTTACT GTACCATA 6900
GCCATATGCC ATATATTAAT CGAATAATCT ATTTAACACA GGGGATCGCA TTTGCTTTCG 6960
NCCCGTTCCG CTTGATATA TATATTTTATA TATAATATTA TATATTTTAC TATTCCTTTCG 7020
GCCATTGCT CGNAAATGCG GCCACTCTCG TTGCTACCAT GTATTCTATA TATTCGATA 7080
TATAATTTA CTAGTGCAAG TACCCCAGGC GATGATACAA ATAAACAGAC CATAATACGG 7140
CGATCGATCG ATCCGGATAT CTATGTATTT ATGTGAGACG CAACTGTGAC CCGTCCACA 7200
CTCGCCGCGG AATTTAGCTGA CCCACGCTAC TCCTTTCCCG TTCCCTCTTT CGACGGTGTG 7260
GTGCCTATT TCCCTTCCTC ATGTAGGACA TTCTNAATCA ATCGAGGCTA TACAGTTTTT 7320
AAGCAAGTCA TTGCAATGCC CAGCCCATCTCG TGAAGTACAC GGGCAACCCG 7380
CCCAGCATGA GTGCGCGAGC AAATACATCT CTCCGATCCC CCCACGATCT GCCATCGGCCG 7440
AGAGAAGCCA GTCGAGGAGC TGCGGAAATTG AACGTAAGAC AGATTTCTTT TGTGATAGA 7500
AAACGAAACA GGTGTTGGGA GATGTTAACC AGAGAAATGT CGAATGACAA AAGATAAATA 7560
ATGATAAAACT AAACCTAAAGT TCTAACAACA ACAACACACA GTAAAATCGC AGAGAACGCG 7620
ACCGATTACA AAATACAAAA ACCTGCAAGC GCCTTTTAAA AACGCTCCGT TCGGTGCTT 7680
CAGAAGAACG AGTAAAGCAT ATAAAGTGCA TAAAGCAAAA CTCTTTTAGT CTAGTTGCTC 7740
AAAGGATATAA GTATTTGGAT AGAACCAGGA AGGATCGAGA ACAAAACCAA TAAACCAACA 7800
AGAATCGAGA TGAATGGTAAG CGAACAAGGC CCCCCACGAC TGTAACGAAAT TCCAAACAAA 7860
TGTTGCAAG TGCTTTTCTTA GAAATTGTCG AAATAGTGCAG AAATGGAACG 7920
GTAACCTAATA TTAATTAATT CCTAATACAA TTGAGAATAAC ATAAAACCTTA ACATAAAACT 7980
GGAAAAAACG ATCTAGCGTG GTAGTGCGA TGTAATATCTC TAACAATTAA CAATTACCGG 8040
CTTAAGTTAG ACCTAAAAAT CGAACAAAAC TCGAAGCGCA TTTAAAGAAA TTAATAAAT 8100
AATATAATTT TTTAGAATCT ATATAATTCT TATATTGATG TGTGTATGTAT GTGAGCCCC 8160
ATATGTATAT GAAAAGGTGG TTTGACTATT TTTGACTATT TTATTTATTA TATATTATAT 8220
ATCGATATAC GATACTGGG TAAATAGCCCC TTTTGGGCTC TTTGAGTGTC TTTTATATAT 8280
ATTTATACG TGTTTATATT TTATAATTTA TTTGACTATA ACTATGGCGA CCAATTAAC 8340
GCATACCTTA TGTATAACCT ATTTGACAAAA AAAAAAAA 8378
(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1480 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: signal sequence
(B) LOCATION: 1 to 36
(C) IDENTIFICATION METHOD: similarity to other signal sequences.
(D) OTHER INFORMATION: Directs Export

(A) NAME/KEY: Four Flank-LRR-Flank domains
(B) LOCATION: 37 to 910
(C) IDENTIFICATION METHOD: Array of Flank-LRR-Flank domains defined herein.
(D) OTHER INFORMATION: mediates adhesive events

(A) NAME/KEY: Tandem EGF-like repeats
(B) LOCATION: 911 to 1150
(C) IDENTIFICATION METHOD: similarity to tandem EGF-like repeats
(D) OTHER INFORMATION: protein-protein interactions

(A) NAME/KEY: 7th EGF-like repeat
(B) LOCATION: 1353 to 1393
(C) IDENTIFICATION METHOD: similarity to epidermal growth factor
(D) OTHER INFORMATION: Involvement in receptor-ligand interactions

(A) NAME/KEY: Alternative splice segment
(B) LOCATION: 1394 to 1404
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: developmentally regulated

(A) NAME/KEY: COOH-terminal region
(B) LOCATION: 1405 to 1480
(C) IDENTIFICATION METHOD: experimental

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

Met Ala Ala Pro Ser Arg Thr Thr Leu Met Pro Pro Pro Phe Arg
5     10    15

Leu Gln Leu Arg Leu Leu Ile Leu Leu Leu Leu Arg
20    25    30

His Asp Ala Val His Ala Glu Pro Tyr Ser Gly Gly Phe Gly Ser
35    40    45

Ser Ala Val Ser Ser Gly Gly Leu Gly Ser Val Gly Ile His Ile
50    55    60

Pro Gly Gly Gly Val Gly Val Ile Thr Glu Ala Arg Cys Pro Arg
65    70    75
Val Cys Ser Cys Thr Gly Leu Asn Val Asp Cys Ser His Arg Gly  
80 85 90
Leu Thr Ser Val Pro Arg Lys Ile Ser Ala Asp Val Glu Arg Leu  
95 100 105
Glu Leu Gln Gly Asn Asn Leu Thr Val Ile Tyr Glu Thr Asp Phe  
110 115 120
Gln Arg Leu Thr Lys Leu Arg Met Leu Gln Leu Thr Asp Asn Gln  
125 130 135
Ile His Thr Ile Glu Arg Asn Ser Phe Gln Asp Leu Val Ser Leu  
140 145 150
Glu Arg Leu Asp Ile Ser Asn Asn Val Ile Thr Thr Val Gly Arg  
155 160 165
Arg Val Phe Lys Gly Ala Gln Ser Leu Arg Ser Leu Gln Leu Asp  
170 175 180
Asn Asn Gln Ile Thr Cys Leu Asp Glu His Ala Phe Lys Gly Leu  
180 185 190
Val Glu Leu Glu Ile Leu Thr Leu Asn Asn Asn Asn Leu Thr Ser  
200 205 210
Leu Pro His Asn Ile Phe Gly Gly Leu Gly Arg Leu Arg Ala Leu  
215 220 225
Arg Leu Ser Asp Asn Pro Phe Ala Cys Asp Cys His Leu Ser Trp  
230 235 240
Leu Ser Arg Phe Leu Arg Ser Ala Thr Arg Leu Ala Pro Tyr Thr  
240 245 250 255
Arg Cys Gln Ser Pro Ser Gln Leu Lys Gly Gln Asn Val Ala Asp  
260 265 270
Leu His Asp Gln Glu Phe Lys Cys Ser Gly Leu Thr Glu His Ala  
275 280 285
Pro Met Glu Cys Gly Ala Glu Asn Ser Cys Pro His Pro Cys Arg  
290 295 300 305
Cys Ala Asp Gly Ile Val Asp Cys Arg Glu Lys Ser Leu Thr Ser  
300 305 310 315
Val Pro Val Thr Leu Pro Asp Thr Thr Asp Val Arg Leu Glu  
320 325 330
Gln Asn Phe Ile Thr Glu Leu Pro Pro Lys Ser Phe Ser Ser Phe  
330 335 340 345
Arg Arg Leu Arg Arg Ile Asp Leu Ser Asn Asn Ile Ser Arg  
350 355 360
<table>
<thead>
<tr>
<th>Residues</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile Ala His Asp Ala Leu Ser Gly Leu Lys Gln Leu Thr Thr Leu</td>
<td>365 370 375</td>
</tr>
<tr>
<td>Val Leu Tyr Gly Asn Lys Ile Lys Asp Leu Pro Ser Gly Val Phe</td>
<td>380 385 390</td>
</tr>
<tr>
<td>Lys Gly Leu Gly Ser Leu Arg Leu Leu Leu Leu Leu Asn Ala Asn Glu</td>
<td>395 400 405</td>
</tr>
<tr>
<td>Ile Ser Cys Ile Arg Lys Asp Ala Phe Arg Asp Leu His Ser Leu</td>
<td>410 415 420</td>
</tr>
<tr>
<td>Ser Leu Leu Ser Leu Tyr Asn Asn Ile Gln Ser Leu Ala Asn</td>
<td>425 430 435</td>
</tr>
<tr>
<td>Gly Thr Phe Asp Ala Met Lys Ser Met Lys Thr Val His Leu Ala</td>
<td>440 445 450</td>
</tr>
<tr>
<td>Lys Asn Pro Phe Ile Cys Asp Cys Asn Leu Arg Trp Leu Ala Asp</td>
<td>455 460 465</td>
</tr>
<tr>
<td>Tyr Leu His Lys Asn Pro Ile Glu Thr Ser Gly Ala Arg Cys Glu</td>
<td>470 475 480</td>
</tr>
<tr>
<td>Ser Pro Lys Arg Met His Arg Arg Arg Ile Glu Ser Leu Arg Glu</td>
<td>485 490 495</td>
</tr>
<tr>
<td>Glu Lys Phe Lys Cys Ser Trp Gly Glu Leu Arg Met Lys Leu Ser</td>
<td>500 505 510</td>
</tr>
<tr>
<td>Gly Glu Cys Arg Met Asp Ser Asp Cys Pro Ala Met Cys His Cys</td>
<td>515 520 525</td>
</tr>
<tr>
<td>Glu Gly Thr Thr Val Asp Cys Thr Gly Arg Arg Leu Lys Glu Ile</td>
<td>530 535 540</td>
</tr>
<tr>
<td>Pro Arg Asp Ile Pro Leu His Thr Thr Glu Leu Leu Leu Leu Asn Asp</td>
<td>545 550 555</td>
</tr>
<tr>
<td>Asn Glu Leu Gly Arg Ile Ser Ser Asp Gly Leu Phe Gly Arg Leu</td>
<td>560 565 570</td>
</tr>
<tr>
<td>Pro His Leu Val Lys Leu Glu Leu Lys Arg Asn Gln Leu Thr Gly</td>
<td>575 580 585</td>
</tr>
<tr>
<td>Ile Glu Pro Asn Ala Phe Glu Gly Ala Ser His Ile Gln Glu Leu</td>
<td>590 595 600</td>
</tr>
<tr>
<td>Gln Leu Gly Glu Asn Lys Ile Lys Glu Ile Ser Asn Lys Met Phe</td>
<td>605 610 615</td>
</tr>
<tr>
<td>Leu Gly Leu His Gln Leu Lys Thr Leu Asn Leu Tyr Asn Gln</td>
<td>620 625 630</td>
</tr>
<tr>
<td>Ile Ser Cys Val Met Pro Gly Ser Phe Glu His Leu Asn Ser Leu</td>
<td>635 640 645</td>
</tr>
</tbody>
</table>
Thr Ser Leu Asn Leu Ala Ser Asn Pro Phe Asn Cys Asn Cys His
650 655 660
Leu Ala Trp Phe Ala Glu Cys Val Arg Lys Lys Ser Leu Asn Gly
665 670 675
Gly Ala Ala Arg Cys Gly Ala Pro Ser Lys Val Arg Asp Val Gln
680 685 690
Ile Lys Asp Leu Pro His Ser Glu Phe Lys Cys Ser Ser Glu Asn
695 700 705
Ser Glu Gly Cys Leu Gly Asp Gly Tyr Cys Pro Pro Ser Cys Thr
710 715 720
Cys Thr Gly Thr Val Val Ala Cys Ser Arg Asn Gln Leu Lys Glu
725 730 735
Ile Pro Arg Gly Ile Pro Ala Glu Thr Ser Glu Leu Tyr Leu Glu
740 745 750
Ser Asn Glu Ile Glu Gln Ile His Tyr Glu Arg Ile Arg His Leu
755 760 765
Arg Ser Leu Thr Arg Leu Asp Leu Ser Asn Gln Ile Thr Ile
770 775 780
Leu Ser Asn Tyr Thr Phe Ala Asn Leu Thr Lys Leu Ser Thr Leu
785 790 795
Ile Ile Ser Tyr Asn Lys Leu Gln Cys Leu Gln Arg His Ala Leu
800 805 810
Ser Gly Leu Asn Asn Leu Arg Val Val Ser Leu His Gly Asn Arg
815 820 825
Ile Ser Met Leu Pro Glu Gly Ser Phe Glu Asp Leu Lys Ser Leu
830 835 840
Thr His Ile Ala Leu Gly Ser Asn Pro Leu Tyr Cys Asp Cys Gly
845 850 855
Leu Lys Trp Phe Ser Asp Trp Ile Lys Leu Asp Tyr Val Glu Pro
860 865 870
Gly Ile Ala Arg Cys Ala Glu Pro Glu Gln Met Lys Asp Lys Leu
875 880 885
Ile Leu Ser Thr Pro Ser Ser Ser Phe Val Cys Arg Gly Arg Val
890 895 900
Arg Asn Asp Ile Leu Ala Lys Cys Asn Ala Cys Phe Glu Gln Pro
905 910 915
Cys Gln Asn Glu Ala Glu Cys Val Ala Leu Pro Gln Arg Glu Tyr
920 925 930
Gln Cys Leu Cys Gln Pro Gly Tyr His Gly Lys His Cys Glu Phe
935  940  945
Met Ile Asp Ala Cys Tyr Gly Asn Pro Cys Arg Asn Asn Ala Thr
950  955  960
Cys Thr Val Leu Glu Glu Gly Arg Phe Ser Cys Gln Cys Ala Pro
965  970  975
Gly Tyr Thr Gly Ala Arg Cys Glu Thr Asn Ile Asp Asp Cys Leu
980  985  990
Gly Glu Ile Lys Cys Gln Asn Asn Ala Thr Cys Ile Asp Gly Val
995 1000 1005
Glu Ser Tyr Lys Cys Glu Cys Gln Pro Gly Phe Ser Gly Glu Phe
1010 1015 1020
Cys Asp Thr Lys Ile Gln Phe Cys Ser Pro Glu Phe Asn Pro Cys
1025 1030 1035
Ala Asn Gly Ala Lys Cys Met Asp His Phe Thr His Tyr Ser Cys
1040 1045 1050
Asp Cys Gln Ala Gly Phe His Gly Thr Asn Cys Thr Asp Asn Ile
1055 1060 1065
Asp Asp Cys Gln Asn His Met Cys Gln Asn Gly Gly Thr Cys Val
1070 1075 1080
Asp Gly Ile Asn Asp Tyr Gln Cys Arg Cys Pro Asp Asp Tyr Thr
1085 1090 1095
Gly Lys Tyr Cys Glu Gly His Asn Met Ile Ser Met Met Tyr Pro
1100 1105 1110
Gln Thr Ser Pro Cys Gln Asn His Glu Cys Lys His Gly Val Cys
1115 1120 1125
Phe Gln Pro Asn Ala Gln Gly Ser Asp Tyr Leu Cys Arg Cys His
1130 1135 1140
Pro Gly Tyr Thr Gly Lys Trp Cys Glu Tyr Leu Thr Ser Ile Ser
1145 1150 1155
Phe Val His Asn Ser Ser Phe Val Glu Leu Glu Pro Leu Arg Thr
1160 1165 1170
Arg Pro Glu Ala Val Thr Ile Val Phe Ser Ser Ala Glu Gln
1175 1180 1185
Asn Gly Ile Leu Met Tyr Asp Gly Gln Asp Ala His Leu Ala Val
1190 1195 1200
Glu Leu Phe Asn Gly Arg Ile Arg Val Ser Tyr Asp Val Gly Asn
1205 1210 1215
<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Start Position</th>
<th>End Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>His Pro Val Ser Thr Met Tyr Ser Phe Glu Met Val Ala Asp Gly</td>
<td>1220</td>
<td>1225</td>
</tr>
<tr>
<td>Lys Tyr His Ala Val Glu Leu Leu Ala Ile Lys Lys Asn Phe Thr</td>
<td>1235</td>
<td>1240</td>
</tr>
<tr>
<td>Leu Arg Val Asp Arg Gly Leu Ala Arg Ser Ile Ile Asn Glu Gly</td>
<td>1250</td>
<td>1255</td>
</tr>
<tr>
<td>Ser Asn Asp Tyr Leu Lys Leu Thr Thr Pro Met Phe Leu Gly Gly</td>
<td>1265</td>
<td>1270</td>
</tr>
<tr>
<td>Leu Pro Val Asp Pro Ala Gln Gln Ala Tyr Lys Asn Trp Gln Ile</td>
<td>1280</td>
<td>1285</td>
</tr>
<tr>
<td>Arg Asn Leu Thr Ser Phe Gly Cys Met Lys Glu Val Trp Ile</td>
<td>1295</td>
<td>1300</td>
</tr>
<tr>
<td>Asn His Lys Leu Val Asp Phe Gly Asn Ala Gln Arg Gln Glu Lys</td>
<td>1310</td>
<td>1315</td>
</tr>
<tr>
<td>Ile Thr Pro Gly Cys Ala Leu Leu Glu Gly Glu Gln Gln Glu Glu</td>
<td>1325</td>
<td>1330</td>
</tr>
<tr>
<td>Glu Asp Asp Glu Gln Asp Phe Met Asp Glu Thr Pro His Ile Lys</td>
<td>1340</td>
<td>1345</td>
</tr>
<tr>
<td>Glu Glu Pro Val Asp Pro Cys Leu Glu Asn Lys Cys Arg Arg Gly</td>
<td>1350</td>
<td>1355</td>
</tr>
<tr>
<td>Ser Arg Cys Val Pro Asn Ser Asn Ala Arg Asp Gly Tyr Glu Cys</td>
<td>1360</td>
<td>1365</td>
</tr>
<tr>
<td>Lys Cys Lys His Gly Gln Arg Gly Arg Tyr Cys Asp Gln Gly Glu</td>
<td>1370</td>
<td>1375</td>
</tr>
<tr>
<td>Gly Ser Thr Glu Pro Pro Thr Val Thr Ala Ala Ser Thr Cys Arg</td>
<td>1380</td>
<td>1385</td>
</tr>
<tr>
<td>Lys Glu Gln Val Arg Glu Tyr Tyr Thr Glu Asn Asp Cys Arg Ser</td>
<td>1390</td>
<td>1395</td>
</tr>
<tr>
<td>Arg Gln Pro Leu Lys Tyr Ala Lys Cys Val Gly Gly Cys Gly Asn</td>
<td>1400</td>
<td>1405</td>
</tr>
<tr>
<td>Gln Cys Ala Ala Lys Ile Val Arg Arg Arg Lys Val Arg Met</td>
<td>1410</td>
<td>1415</td>
</tr>
<tr>
<td>Val Cys Ser Asn Asn Arg Lys Tyr Ile Lys Asn Leu Arg Ile Val</td>
<td>1420</td>
<td>1425</td>
</tr>
<tr>
<td>Arg Lys Cys Gly Cys Thr Lys Lys Cys Tyr</td>
<td>1435</td>
<td>1440</td>
</tr>
<tr>
<td></td>
<td>1445</td>
<td>1450</td>
</tr>
<tr>
<td></td>
<td>1455</td>
<td>1460</td>
</tr>
<tr>
<td></td>
<td>1465</td>
<td>1470</td>
</tr>
<tr>
<td></td>
<td>1475</td>
<td>1480</td>
</tr>
</tbody>
</table>
(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 222 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Flank-LRR-Flank 1
(B) LOCATION: 1 to 222
(C) IDENTIFICATION METHOD: similarity to other Flank-LRR-
Flank domains defined herein.
(D) OTHER INFORMATION: mediates adhesive events

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

Cys Pro Arg Val Cys Ser Cys Thr Gly Leu Asn Val Asp Cys Ser  
5              10
His Arg Gly Leu Thr Ser Val Pro Arg Lys Ile Ser Ala Asp Val  
20             25
Glu Arg Leu Glu Leu Gln Gly Asn Leu Thr Val Ile Tyr Glu  
35             40
Thr Asp Phe Gln Arg Leu Thr Lys Leu Arg Met Leu Gln Leu Thr  
50             55
Asp Asn Gln Ile His Thr Ile Glu Arg Asn Ser Phe Gln Asp Leu  
65             70
Val Ser Leu Glu Arg Leu Asp Ile Ser Asn Asn Val Ile Thr Thr  
80             85
Val Gly Arg Val Phe Lys Gly Ala Gln Ser Leu Arg Ser Leu  
95             100
Gln Leu Asp Asn Gln Ile Thr Cys Leu Asp Glu His Ala Phe  
110            115
Lys Gly Leu Val Glu Leu Glu Ile Leu Thr Leu Asn Asn Asn  
125            130
Leu Thr Ser Leu Pro His Asn Ile Phe Gly Gly Leu Gly Arg Leu  
140            145
Arg Ala Leu Arg Leu Ser Asp Asn Pro Phe Ala Cys Asp Cys His  
155            160
Leu Ser Trp Leu Ser Arg Phe Leu Arg Ser Ala Thr Arg Leu Ala  
170            175
Pro Tyr Thr Arg Cys Gln Ser Pro Ser Gln Leu Lys Gly Gln Asn  
185            190
Val Ala Asp Leu His Asp Gln Glu Phe Lys Cys Ser Gly Leu Thr  
200            205
Glu His Ala Pro Met Glu Cys Gly Ala Glu Asn Ser  
215            220
(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 224 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Flank-LRR-Flank 2
(B) LOCATION: 1 to 224
(C) IDENTIFICATION METHOD: similarity to other Flank-LRR-Flank domains defined herein.
(D) OTHER INFORMATION: mediates adhesive events

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

Cys Pro His Pro Cys Arg Cys Ala Asp Gly Ile Val Asp Cys Arg
5    10    15

Glu Lys Ser Leu Thr Ser Val Pro Val Thr Leu Pro Asp Thr
20   25    30

Thr Asp Val Arg Leu Glu Gln Asn Phe Ile Thr Glu Leu Pro Pro
35   40    45

Lys Ser Phe Ser Ser Phe Arg Arg Leu Arg Arg Ile Asp Leu Ser
50   55    60

Asn Asn Ile Ser Arg Ile Ala His Asp Ala Leu Ser Gly Leu
65   70    75

Lys Gln Leu Thr Leu Val Leu Tyr Gly Asn Lys Ile Lys Asp
80   85    90

Leu Pro Ser Gly Val Phe Lys Gly Leu Gly Ser Leu Arg Leu Leu
95   100   105

Leu Leu Asn Ala Asn Glu Ile Ser Cys Ile Arg Lys Asp Ala Phe
110  115   120

Arg Asp Leu His Ser Leu Ser Leu Ser Leu Tyr Asp Asn Asn
125  130   135

Ile Gln Ser Leu Ala Asn Gly Thr Phe Asp Ala Met Lys Ser Met
140  145   150

Lys Thr Val His Leu Ala Lys Asn Pro Phe Ile Cys Asp Cys Asn
155  160   165

Leu Arg Trp Leu Ala Asp Tyr Leu His Lys Asn Pro Ile Glu Thr
170  175   180

Ser Gly Ala Arg Cys Glu Ser Pro Lys Arg Met His Arg Arg Arg
185  190   195

Ile Glu Ser Leu Arg Glu Lys Phe Lys Cys Ser Trp Gly Glu
200  205   210

Leu Arg Met Lys Leu Ser Gly Glu Cys Arg Met Asp Ser Asp
215  220
(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Flank-LRR-Flank 3
(B) LOCATION: 1 to 196
(C) IDENTIFICATION METHOD: similarity to other Flank-LRR-
Flank domains defined herein.
(D) OTHER INFORMATION: mediates adhesive events

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

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

Tyr
(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 196 amino acids
   (B) TYPE: amino acids
   (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
   (A) NAME/KEY: Flank-LRR-Flank 4
   (B) LOCATION: 1 to 196
   (C) IDENTIFICATION METHOD: similarity to other Flank-LRR-
   Flank domains defined herein.
   (D) OTHER INFORMATION: mediates adhesive events

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

<table>
<thead>
<tr>
<th></th>
<th>Cys</th>
<th>Pro</th>
<th>Pro</th>
<th>Ser</th>
<th>Cys</th>
<th>Thr</th>
<th>Cys</th>
<th>Thr</th>
<th>Gly</th>
<th>Thr</th>
<th>Val</th>
<th>Val</th>
<th>Ala</th>
<th>Cys</th>
<th>Ser</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Arg</th>
<th>Asn</th>
<th>Gln</th>
<th>Leu</th>
<th>Lys</th>
<th>Glu</th>
<th>Ile</th>
<th>Pro</th>
<th>Arg</th>
<th>Gly</th>
<th>Ile</th>
<th>Pro</th>
<th>Ala</th>
<th>Glu</th>
<th>Thr</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ser</th>
<th>Glu</th>
<th>Leu</th>
<th>Tyr</th>
<th>Leu</th>
<th>Glu</th>
<th>Ser</th>
<th>Asn</th>
<th>Glu</th>
<th>Ile</th>
<th>Glu</th>
<th>Gln</th>
<th>Ile</th>
<th>His</th>
<th>Tyr</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Glu</th>
<th>Arg</th>
<th>Ile</th>
<th>Arg</th>
<th>His</th>
<th>Leu</th>
<th>Arg</th>
<th>Ser</th>
<th>Leu</th>
<th>Thr</th>
<th>Arg</th>
<th>Leu</th>
<th>Asp</th>
<th>Leu</th>
<th>Ser</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Asn</th>
<th>Asn</th>
<th>Gln</th>
<th>Ile</th>
<th>Thr</th>
<th>Leu</th>
<th>Ser</th>
<th>Asn</th>
<th>Tyr</th>
<th>Thr</th>
<th>Phe</th>
<th>Ala</th>
<th>Asn</th>
<th>Leu</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Thr</th>
<th>Lys</th>
<th>Leu</th>
<th>Ser</th>
<th>Thr</th>
<th>Leu</th>
<th>Ile</th>
<th>Ile</th>
<th>Ser</th>
<th>Tyr</th>
<th>Asn</th>
<th>Lys</th>
<th>Leu</th>
<th>Gln</th>
<th>Cys</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Leu</th>
<th>Gln</th>
<th>Arg</th>
<th>His</th>
<th>Ala</th>
<th>Leu</th>
<th>Ser</th>
<th>Gly</th>
<th>Leu</th>
<th>Asn</th>
<th>Asn</th>
<th>Leu</th>
<th>Arg</th>
<th>Val</th>
<th>Val</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ser</th>
<th>Leu</th>
<th>His</th>
<th>Gly</th>
<th>Asn</th>
<th>Arg</th>
<th>Ile</th>
<th>Ser</th>
<th>Met</th>
<th>Leu</th>
<th>Pro</th>
<th>Glu</th>
<th>Gly</th>
<th>Ser</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>115</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Glu</th>
<th>Asp</th>
<th>Leu</th>
<th>Ser</th>
<th>Leu</th>
<th>Thr</th>
<th>His</th>
<th>Ile</th>
<th>Ala</th>
<th>Leu</th>
<th>G1y</th>
<th>Ser</th>
<th>Asn</th>
<th>Pro</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Leu</th>
<th>Tyr</th>
<th>Cys</th>
<th>Asp</th>
<th>Cys</th>
<th>Gly</th>
<th>Leu</th>
<th>Lys</th>
<th>Trp</th>
<th>Phe</th>
<th>Ser</th>
<th>Asp</th>
<th>Trp</th>
<th>Ile</th>
<th>Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>145</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Leu</th>
<th>Asp</th>
<th>Tyr</th>
<th>Val</th>
<th>Glu</th>
<th>Pro</th>
<th>Gly</th>
<th>Ile</th>
<th>Ala</th>
<th>Arg</th>
<th>Cys</th>
<th>Ala</th>
<th>Glu</th>
<th>Pro</th>
<th>Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Gln</th>
<th>Met</th>
<th>Lys</th>
<th>Asp</th>
<th>Lys</th>
<th>Leu</th>
<th>Leu</th>
<th>Ser</th>
<th>Thr</th>
<th>Pro</th>
<th>Ser</th>
<th>Ser</th>
<th>Ser</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>175</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Val</th>
<th>Cys</th>
<th>Arg</th>
<th>Gly</th>
<th>Arg</th>
<th>Val</th>
<th>Arg</th>
<th>Asp</th>
<th>Ile</th>
<th>Leu</th>
<th>Ala</th>
<th>Lys</th>
<th>Cys</th>
<th>Asn</th>
</tr>
</thead>
<tbody>
<tr>
<td>185</td>
<td>190</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ala
(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 11 amino acids
   (B) TYPE: amino acids
   (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: Internal fragment
(ix) FEATURE:
   (A) NAME/KEY: Alternate segment
   (B) LOCATION: 1 to 11
   (C) IDENTIFICATION METHOD: Experimental

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

Gly Glu Gly Ser Thr Glu Pro Pro Thr Val Thr
  5                   10
(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: Yes

(ix) FEATURE:
(A) NAME/KEY: Flank-LRR-Flank consensus
(B) LOCATION: 1 to 196
(C) IDENTIFICATION METHOD: Experimental

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

Cys Pro Xaa Xaa Cys Xaa Cys Xaa Gly Xaa Xaa Val Asp Cys Xaa
   5    10   15
Xaa Xaa Glu Leu Xaa Xaa Xaa Pro Xaa Xaa Xaa Pro Xaa Asp Thr
   20   25   30
Thr Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Ile Xaa Xaa Leu Xaa Xaa
   35   40   45
Xaa Xaa Phe Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Leu Xaa Leu Xaa
   50   55   60
Xaa Asn Xaa Ile Xaa Xaa Leu Xaa Xaa Asn Xaa Ile Xaa Xaa
   65   70   75
Xaa Xaa Leu Xaa Xaa Leu Ile Leu Xaa Xaa Asn Xaa Ile Xaa Xaa
   80   85   90
Leu Xaa Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Leu
   95   100  105
Xaa Leu Xaa Xaa Asn Xaa Ile Xaa Xaa Leu Xaa Xaa Xaa Xaa Phe
  110   115  120
Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Leu Xaa Leu Xaa Xaa Asn Pro
  125   130  135
Phe Xaa Cys Asp Cys Xaa Leu Xaa Trp Leu Xaa Xaa Xaa Xaa
  140   145  150
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Arg Cys Xaa Xaa Pro Leu
  155   160  165
Xaa Xaa Xaa Xaa Xaa Ile Xaa Xaa Leu Xaa Xaa Xaa Phe
  170   175  180
Lys Cys Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
  185   190  195
(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 76 amino acids
   (B) TYPE: amino acids
   (C) TOPOLOGY: Linear
   (D) MOLECULE TYPE: protein

(ix) FEATURE:
   (A) NAME/KEY: COOH terminal region
   (B) LOCATION: 1 to 76
   (C) IDENTIFICATION METHOD: Experimental

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

<table>
<thead>
<tr>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
<th>65</th>
<th>70</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Ala</td>
<td>Ser</td>
<td>Thr</td>
<td>Cys</td>
<td>Arg</td>
<td>Lys</td>
<td>Glu</td>
<td>Gln</td>
<td>Val</td>
<td>Arg</td>
<td>Glu</td>
<td>Tyr</td>
<td>Tyr</td>
<td>Thr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Asn</td>
<td>Asp</td>
<td>Cys</td>
<td>Arg</td>
<td>Ser</td>
<td>Arg</td>
<td>Glu</td>
<td>Gln</td>
<td>Pro</td>
<td>Leu</td>
<td>Lys</td>
<td>Tyr</td>
<td>Ala</td>
<td>Lys</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Gly</td>
<td>Gly</td>
<td>Cys</td>
<td>Gly</td>
<td>Asn</td>
<td>Gln</td>
<td>Cys</td>
<td>Cys</td>
<td>Ala</td>
<td>Ala</td>
<td>Lys</td>
<td>Ile</td>
<td>Val</td>
<td>Arg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>Arg</td>
<td>Lys</td>
<td>Val</td>
<td>Arg</td>
<td>Met</td>
<td>Val</td>
<td>Cys</td>
<td>Ser</td>
<td>Asn</td>
<td>Asn</td>
<td>Arg</td>
<td>Lys</td>
<td>Tyr</td>
<td>Ile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Asn</td>
<td>Leu</td>
<td>Asp</td>
<td>Ile</td>
<td>Val</td>
<td>Arg</td>
<td>Lys</td>
<td>Cys</td>
<td>Gly</td>
<td>Cys</td>
<td>Thr</td>
<td>Lys</td>
<td>Lys</td>
<td>Cys</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tyr</td>
</tr>
</tbody>
</table>

Tyr
WHAT IS CLAIMED IS:

1. An isolated and substantially pure form of the SLIT protein comprising the sequence SEQ I.D. NO. 2.

2. An isolated DNA segment encoding the entire SLIT protein, SEQ. I.D. NO. 1.

3. A recombinant expression vector comprising the DNA segment according to claim 2.

4. A recombinant host microorganism containing a DNA expression vector comprising a DNA sequence consisting essentially of a DNA sequence encoding the entire SLIT protein.

5. An amino flank-LRR-carboxy-flank consensus sequence element of the SLIT protein (SEQ. I.D. NO. 8) comprising
   (a) an amino-flanking region comprising the sequence CPxxCxC........xGxxVDCxxxGLx...xαPxxαPxDTTx,
   (b) a leucine-rich repeat region comprising one or more repeats of the sequence xxxxFxXXLxxLxxNxIxxL, and
   (c) a carboxy-flanking region comprising the sequence P(W or F)xC(D or N)Cxα.....W(L or F)xxxxxxxxx.............RCxxPxxxxxxaxxxaxxxxFx..C (P or S).

6. The first amino-flank-LRR-carboxy-flank sequence element of the SLIT protein (SEQ. I.D. NO. 3) according to claim 5 wherein
   (a) the amino-flanking region comprises the sequence CPRVCSC TGLNVDCSHRLT SVPRKISADVER,
   (b) the leucine-rich region comprises the sequence
and

c) the carboxy-flanking region comprises the sequence
PPACD CHL SWLSRFLRSAATRLAPYT RCQSPQLKGQNVADLHDQEFK
CGLTEHAPMECGAENS.

7. The second amino-flank-LRR-carboxy-flank sequence
element of the SLIT protein (SEQ. I.D. NO. 4) according to
claim 5 wherein
(a) the amino-flanking region comprises the sequence
CPHPCRC ADGIVDCREKSLT SVPVTLPDDTD,
(b) the leucine-rich region comprises the sequence
VRLECPFTEL
PPKSFSSFFRLARIDLSMNISRI
AHDLALSGLQILTVLYQMKIKDL
PSGVKFLSGSLRLLLLANEMISC
RKAFAFLNLSLSSLHLYMNNIQSL
ANGTFLDANKSMKTVLHAPH

and

c) the carboxy-flanking region comprises the sequence
PFICCNCL RWLADLYHLKIPETSGARCESPKRMHRRIESLREEFK
CSWGEILRMKLSGECRMDSD.

8. The third amino-flank-LRR-carboxy-flank sequence
element of the SLIT protein (SEQ. I.D. NO. 5) according to
claim 5, wherein
(a) the amino-flanking region comprises the sequence
CPAMCHC EGTTVDCCTGRGLK EIPRDIPLHTTE
(b) the leucine-rich repeat region comprises the sequence
LLMMINTERNLSR
SDGLFGRLPHLVEKLELPMNQIXI
EPNAFEASHIQELQLOEGRIKIK
SNKMFGLHLKTLNLQMQICSV
MFGSFEHLNLSLNLASN

and
(c) the carboxy flanking region comprises the sequence
PFNCNCHLVWFAECVRKKSLLNGGAA RCAPSKVRDVQIKDLPH SEEK
CSSENSEGCLGD GY.

9. The fourth amino-flank-LRR-carboxy-flank sequence
element of the SLIT protein (SEQ. I.D. NO. 6) according to
claim 5, wherein
(a) the amino-flanking region comprising the sequence
CPPSCTC TGTVVACSRNLK EIPRGIPAETSE,
(b) the leucine-rich repeat region comprising the sequence

\[
\begin{align*}
\text{LYLESNIEQI} \\
\text{HYERIRHLSLTRLDLVQITIL} \\
\text{SNYTFANLKLSTLIISYNLKLCL} \\
\text{QHHSILSLAVLVSSLNLRSSML} \\
\text{PESSEFDKSLHTIALGN} \\
\end{align*}
\]

and
(c) the carboxy-flanking region comprising the sequence
PLYCDGGL KWFSDWIKLDYVEFGIA RCAEPQMKDKLILSTPSSSFV
CRGRVRNDILAKCNA.

10. The alternate splice segment of the SLIT protein
residing at the seventh epidermal growth factor (EGF) sequence
element of the SLIT protein comprising the sequence
GEGSTEPFTVT (SEQ. I.D. NO. 7).

11. The carboxy terminal region of the SLIT protein
(SEQ. I.D. NO. 9) residing after the seventh epidermal growth
factor.

12. A combination comprising one or more amino-flank-
LRR-carboxy-flank sequence elements according to claim 5 and
one or more EGF-like repeat elements of the SLIT protein,
provided that said combination does not include the naturally
occurring configuration of the SLIT protein.
13. The combination according to claim 12, further including the alternative splice segment of the SLIT protein residing at the seventh epidermal growth factor sequence element when part of the SLIT protein comprising the sequence GEGSTEPTVT (SEQ. I.D. NO. 7).

14. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 1 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.

15. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 5 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.

16. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 6 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.

17. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 7 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.

18. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 8 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.

19. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 9 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.
20. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 10 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.

21. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 11 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.

22. An antibody to the SLIT protein produced by introducing into an animal the SLIT protein according to claim 12 sufficient to raise antibodies in said animal and withdrawing said antibodies from said animal.

23. A method of detecting the SLIT protein or a shed portion thereof in a bodily fluid comprising contacting the bodily fluid with antibodies raised to the SLIT protein according to claim 1 or to a portion thereof and detecting for the presence of the SLIT protein.

24. A method of detecting autoimmune antibodies to the SLIT protein or a shed portion thereof in a bodily fluid comprising contacting the bodily fluid with the SLIT protein according to claim 1 or a portion thereof and detecting for the presence of said autoimmune antibodies.

25. A method of detecting chromosomal rearrangements in the SLIT locus comprising hybridizing a nucleic acid from a patient with a nucleic acid sequence from the SLIT locus and detecting for the level of expression or an aberrant rearrangement, said nucleic acid sequence being the DNA according to claim 2 or a portion thereof.
26. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 1 or a portion thereof, in admixture with a pharmaceutically acceptable carrier.

27. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 5, in admixture with a pharmaceutically acceptable carrier.

28. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 6, in admixture with a pharmaceutically acceptable carrier.

29. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 7, in admixture with a pharmaceutically acceptable carrier.

30. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 8, in admixture with a pharmaceutically acceptable carrier.
31. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 9, in admixture with a pharmaceutically acceptable carrier.

32. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 10, in admixture with a pharmaceutically acceptable carrier.

33. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 11, in admixture with a pharmaceutically acceptable carrier.

34. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of the SLIT protein according to claim 12, in admixture with a pharmaceutically acceptable carrier.

35. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 1, or a portion thereof, either alone or in admixture with a pharmaceutically acceptable carrier.
36. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 5, either alone or in admixture with a pharmaceutically acceptable carrier.

37. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 6, either alone or in admixture with a pharmaceutically acceptable carrier.

38. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 7, either alone or in admixture with a pharmaceutically acceptable carrier.

39. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 8, either alone or in admixture with a pharmaceutically acceptable carrier.

40. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 9, either alone or in admixture with a pharmaceutically acceptable carrier.
41. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 10, either alone or in admixture with a pharmaceutically acceptable carrier.

42. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 11, either alone or in admixture with a pharmaceutically acceptable carrier.

43. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of the SLIT protein according to claim 12, either alone or in admixture with a pharmaceutically acceptable carrier.

44. A protein, TAGON, that allows for the formation of a molecular bridge between axonally associated receptors and extracellular matrix molecules.

45. A pharmaceutical preparation for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or for affecting the angiogenic process in a patient comprising a sterile preparation comprising an effective amount of a TAGON protein in admixture with a pharmaceutically acceptable carrier.

46. A method for the treatment of a neurodegenerative disease, for treating a traumatic injury to a neural tissue or
for affecting the angiogenic process in a patient comprising administering to said patient an effective amount of a TAGON protein, either alone or in admixture with a pharmaceutically acceptable carrier.
signal peptide

NH₂

FIG. 2A
| Amino-Flanking Region                  |
| Leucine-Rich Repeat (LRR)             |
| Carboxy-Flanking Region               |
| Flank-LRR-Flank                       |
| EGF-Like Repeat                       |
| 11 a.a. Alternate Segment             |

**FIG. 2B**
# INTERNATIONAL SEARCH REPORT

## I. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both National Classification and IPC

<table>
<thead>
<tr>
<th>IPC (S):</th>
<th>C07K 13/00</th>
</tr>
</thead>
<tbody>
<tr>
<td>US CL:</td>
<td>530/350</td>
</tr>
</tbody>
</table>

## II. FIELDS SEARCHED

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Classification Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S.</td>
<td>530/350</td>
</tr>
</tbody>
</table>

Documentation searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched

APS, Medline
search terms: slit protein, Rothberg JM, neuron? (p)adhesion

## III. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>y</td>
<td>Cell, Volume 55, issued 22 December 1988, J.M. Rothberg et al., &quot;slit: An EGF-homologous locus of D. melanogaster involved in the development of the embryonic central nervous system,&quot; pages 1047-1059, see entire contents.</td>
<td>1</td>
</tr>
</tbody>
</table>

- 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
- 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
- Z: document member of the same patent family

## IV. CERTIFICATION

Date of Actual Completion of the International Search: 26 MARCH 1992

Date of Mailing of the International Search Report: 08 APR 1992

International Searching Authority: ISA/US

Signature of Authorized Officer: Michael P. Woodward
VI. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

I. Claim 1, drawn to isolated slit protein, classified in Class 530, subclass 350.
II. Claims 2-4 drawn to an isolated DNA sequence, an expression vector and transformed host cell, classified in Class 536, subclass 27 and 435, subclass 320.1 respectively.
III. Claims 5-13, drawn to peptides, classified in Class 530, subclass 350 and Class 530, subclass 324 or 327.
IV. Claims 14-22, drawn to antibodies, classified in Class 530, subclass 387.
V. Claims 26-34, drawn to pharmaceutical compositions, classified in Class 514, subclass 12.
VI. Claims 35-43, drawn to treatment methods involving peptides, classified in Class 514, subclass 2.
VII. Claim 23, drawn to an immunoassay for slit protein, classified in Class 435, subclass 7.1.
VIII. Claim 24, drawn to an immunocassay for anti-slit protein antibodies, classified in Class 435, subclass 7.1.
IX. Claim 25, drawn to a hybridization assay, classified in Class 435, subclass 6.
X. Claim 44, drawn to tagon protein, classified in Class 530, subclass 350.
XI. Claim 45, drawn to a pharmaceutical composition containing tagon, classified in Class 514, subclass 2.
XII. Claim 46, drawn to a method of treatment using tagon protein, classified in Class 514, subclass 2.

The claims of these twelve groups are drawn to distinct inventions which are not linked so as to form a single general inventive concept. PCT rules 13.1 and 13.2 do not provide for multiple products and methods. The claims of groups III-VI have been grouped using PCT Rule 13.3.