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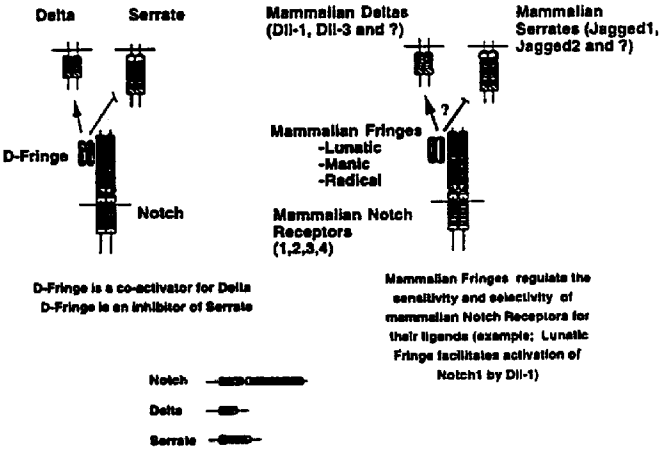
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(54) **PROTEINES "FRINGE" ET SIGNALISATION DE NOTCH**
(54) **FRINGE PROTEINS AND NOTCH SIGNALLING**

Model for Fringe function;
Fringe modifies the Specificity of Notch for its ligands



(57) On a cloné et séquencé trois ADNc frangés de mammifère; on a décrit leur interaction avec la voie de signalisation du récepteur de Notch. L'invention propose des méthodes de prévention et de traitement de troubles caractérisés par une anomalie de signalisation du récepteur de Notch.

(57) Three mammalian Fringe cDNAs have been cloned and sequenced, and their interaction with the Notch receptor signalling pathway has been described. Methods are provided for preventing or treating disorders characterised by an abnormality in Notch receptor signalling.

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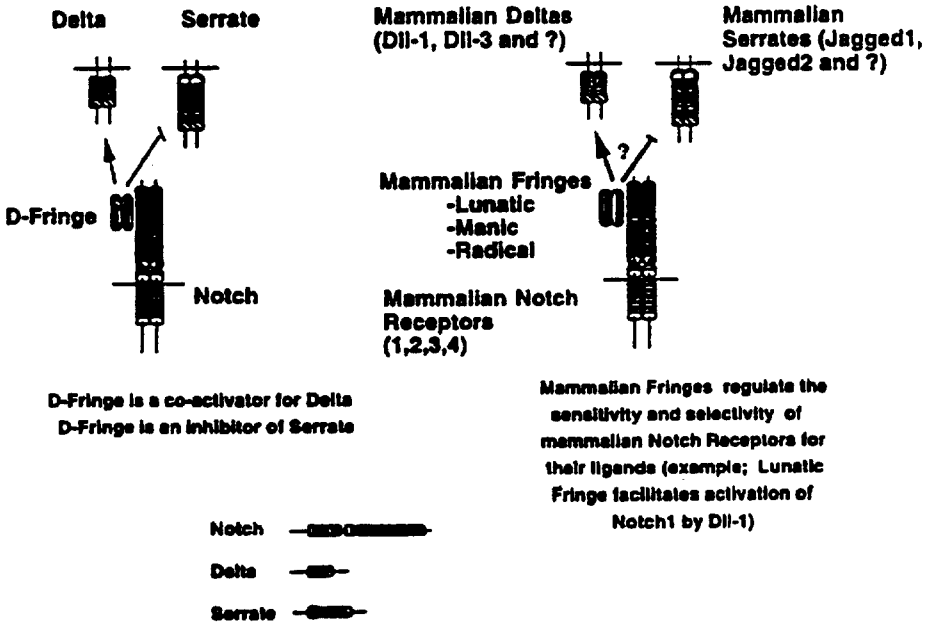
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(57) Abstract

Three mammalian Fringe cDNAs have been cloned and sequenced, and their interaction with the Notch receptor signalling pathway has been described. Methods are provided for preventing or treating disorders characterised by an abnormality in Notch receptor signalling.

Model for Fringe function;
Fringe modifies the Specificity of Notch for its ligands



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FRINGE PROTEINS AND NOTCH SIGNALLING

Field of the Invention

This invention relates to control of the interaction between Notch receptors and their ligands.

Background of the Invention

Various journal articles referred to herein are identified by authors and date in parentheses and are listed, with full citations, at the end of the specification. The contents thereof are incorporated herein by reference.

Genetic analysis of *Drosophila melanogaster* wing development has elucidated many fundamental concepts of tissue induction and specification. These studies have highlighted the importance of compartment boundaries in organizing pattern (Basler and Struhl, 1994; Garcia-Bellido et al., 1973; Lawrence and Morata, 1976; Tabata and Kornberg, 1994).

The wing imaginal disc is divided into anterior/posterior (A/P) compartments and dorso/ventral (D/V) compartments, which are specified during embryogenesis and second larval instar, respectively. The posterior compartment cells express the secreted protein Hedgehog (HH) but do not express the Zn-finger protein Cubitus interruptus (CI) which is required for cells to respond to HH (Dominguez et al., 1996). In contrast, the anterior compartment cells express Ci but no HH. Because the posterior cells express HH but cannot respond to it, whereas the Ci-expressing anterior cells can respond to HH but do not make it, HH response only occurs at the boundary between posterior and anterior compartments (Dominguez et al., 1996) and the HH signalling system is activated at the A/P boundary.

The Notch signalling system is activated by the juxtaposition of the dorsal and ventral compartments (de Celis et al., 1996; Diaz-Benjumea and Cohen, 1993). Establishment of the dorso/ventral compartment boundary is also initiated through the restricted expression of a transcription factor. Dorsal cells express the LIM

domain/homeodomain-containing protein, Apterous (Diaz-Benjumea and Cohen, 1993). Apterous induces expression of the secreted protein Fringe (Irvine and Wieschaus, 1994). Consequently, Fringe is expressed dorsally, whereas the secreted protein Wingless is expressed ventrally (Ng et al., 1996).

Two Notch ligands are also expressed in the wing imaginal disc. Serrate is most strongly expressed in dorsal cells (Kim et al., 1995), and Delta in ventral cells (Doherty et al., 1996). The juxtaposition of dorsal cells expressing Fringe and Serrate with ventral cells which express Wingless and Delta results in the localized activation of Notch on either side of the D/V compartment boundary (de Celis et al., 1996; Irvine and Wieschaus, 1994; Kim et al., 1995). The activated Notch receptor then signals the induction of wing margin tissue at this boundary (de Celis et al., 1996; Diaz-Benjumea and Cohen, 1995; Doherty et al., 1996; Rulifson and Blair, 1995).

It is not understood how Fringe, Wingless, Serrate and Delta proteins induce the activation of Notch at the D/V boundary but not elsewhere in the wing pouch. It is, however, known that Serrate can only activate Notch in ventral cells (Kim et al., 1995) whereas Delta can only activate Notch in dorsal cells (Doherty et al., 1996). In addition, ectopic expression of Fringe in ventral cells causes the local activation of Notch with resulting induction of margin tissue and wing outgrowth (Irvine and Wieschaus, 1994; Kim et al., 1995). Removal of Fringe from dorsal cells has a similar effect (Irvine and Wieschaus, 1994). Both of these phenomena have implicated Fringe in creating boundaries and in controlling Notch activation (Irvine and Wieschaus, 1994).

The precise mechanism by which Fringe regulates Notch activation at the D/V boundary in developing wing discs remains to be elucidated. It has been demonstrated that Fringe boundaries can upregulate Serrate protein

expression in the wing disc (Kim et al., 1995). How this occurs, as well as the potential participation of Delta (Doherty et al., 1996) and Wingless (Couso and Martinez Arias, 1994) in ectopic margin induction by Fringe remains to be investigated. In addition, the roles of Fringe or Fringe related proteins (Wu et al., 1996) in other developmental contexts are also unknown.

Summary of Drawings

Certain embodiments of the invention are described, reference being made to the accompanying drawings, wherein:

Figures 1A and 1B show ectopic expression of Manic and Radical Fringe in Drosophila. Figure 1A shows panels (A) wild type wing; D) eye from GAL4^{ptc} fly (similar to wild type); (B) and (E) wing and eye from flies crossed to UAS- Manic Fringe and GAL4 driver; (C) and (F) wing and eye from flies crossed to UAS-Radical Fringe and GAL4 drivers. B, C, E, F : ectopic expression with GAL4^{ptc} driver. H, I : ectopic expression with GAL 4^{c5} driver. Figure 1B shows panels (G), (H) wings from flies crossed to VAS-Manic Fringe and GAL4 drivers (ectopic expression with GAL4 ^{c96} and GAL4 ^{c5} drivers respectively.

Figure 2 shows wings ectopically expressing *Radical Fringe* with GAL4^{ptc} driver in different genetic backgrounds(A) GAL4^{ptc}/+; UAS-*Radical Fringe*/+ wing(B, C) *fng*^{52}/+ and GAL4^{ptc}/+; UAS-Radical Fringe/*fng*^{52} respectively; (D,H) *Dl*^{x}/+ and GAL4^{ptc}/+; UAS-Radical Fringe/*Dl*^{x} respectively; (E, I) *Ser*^{Rx106}/+ and GAL4^{ptc}/+; UAS-Radical Fringe/*Ser*^{Rx106} respectively; (F, J) Df(1) *N*^{8}/+ and GAL4^{ptc}/+; UAS-Radical Fringe/Df(1) *N*^{8} respectively; (G, K) *wg*^{CX2}/+ and GAL4^{ptc}/+; UAS-Radical Fringe/*wg*^{CX2} respectively;

Figure 3 shows Northern blot analysis of *Fringe* gene expression in mice. Probe detects polyA+ RNA from whole mouse embryos at the indicated stages of development (eg. E7=7 day embryo) hybridized with each of the three mouse

Fringe genes.

Figure 4 shows Northern blot analysis of *Fringe* gene expression in mice. Probe detects RNA from adult mouse tissues hybridized with each of the three mouse *Fringe* genes.

Figures 5A and 5B show expression of mouse Fringe genes in embryonic and selected adult tissues. Figure 5A shows panels (A and B) Whole mount *in situ* hybridization with Lunatic *Fringe* antisense riboprobes in E8.5 and E9.5 day embryos respectively; (C and E) Dark field section *in situ* hybridization with *Lunatic Fringe* antisense riboprobes in E11.5 and E12.5 day embryos respectively; (D) Bright field of E12.5 section shown in panel E (F) Dark field section *in situ* hybridization with *Radical Fringe* antisense riboprobes in E12.5 day embryo

Figure 5B shows panels (G) Bright field of section *in situ* hybridization with *Lunatic Fringe* antisense riboprobes in E13.5 day embryo with close up of grains on S-shaped bodies in kidney; (H and J) Dark field section *in situ* hybridization with *Lunatic Fringe* antisense riboprobes in adult thymus and spleen respectively (I) Bright field of spleen section shown in panel J (K and L) Dark field and bright field of section *in situ* hybridization with *Manic Fringe* antisense riboprobes in adult spleen, with close up of grains in megakaryocytes shown in panel L.

Figure 6 shows *Fringe* gene switch in differentiation in the mouse. (A, B, C) Dark field section *in situ* hybridization with antisense probes to *Lunatic* (A), *Manic* (B) and *Radical* (C) *Fringe* genes in E10.5 mouse embryo neural tubes. vz is the ventricular zone and mz is the marginal zone of the neural tube. (D, E, F) Dark field section in situ hybridization with antisense probes to *Lunatic* (D), *Manic* (E) and *Radical* (F) *Fringe* genes in adult tongue. be is the basal epithelium and sbe is the suprabasal

epithelium.

Figure 7 shows expression of mouse. Notch ligands and *Lunatic Fringe* during somitogenesis and neural tube patterning.

(A, B, C) Whole mount *in situ* hybridization with antisense probes to *Deltal*(A), *Lunatic Fringe*(B) and *Serratel*(C) genes in E8.5 mouse embryo posterior mesoderm. arrowhead points to a forming somite. (D, E, F) Dark field section in situ hybridization with antisense probes to *Deltal*(A), *Lunatic Fringe*(B) and *Serratel*(C) genes in E10.5 mouse embryo neural tube.

Figure 8 shows a schematic diagram of the proposed model for Fringe proteins as regulators of Notch specificity and sensitivity for its ligands.

Figure 9 shows a schematic diagram for the model of Figure 8 applied to the development of wing margin in *Drosophila*.

Detailed Description of Invention

The interaction of Notch receptors with Notch ligands plays an important role in development in mammals and in insects. Activation of a Notch receptor by a Notch ligand initiates signal transduction, the signal being communicated to the cell via the cytosolic domain of the Notch receptor protein.

Notch ligands which activate the Notch receptor and initiate signal transduction include the DSL group of ligands, for example, Delta protein, Serrate protein and Lag-2 protein.

The inventors have cloned and characterized three novel mammalian genes which are related to *Drosophila* Fringe, as described in the Examples herein. These mammalian genes are expressed in tissues which are undergoing Notch-dependent development and differentiation.

Experiments in *Drosophila* with these mammalian *fringe* genes revealed that the Fringe proteins control or modulate activation of the Notch receptor by Notch ligands. The Fringe system of proteins can be used to

induce new cell fates at tissue boundaries, to reinforce predetermined tissue boundaries and to block Notch signalling in differentiating cells.

Mammals have at least four Notch receptors which can interact with Notch ligands (Egan et al. (1997), Current Topics in Microbiology and Immunology, 228, 273 - 324).

The three mammalian Fringe proteins, Lunatic Fringe, Manic Fringe and Radical Fringe, act to promote or inhibit the interaction of Notch receptors with Notch ligands.

The cDNA sequences of murine *Lunatic Fringe* (Sequence ID:NO:1), *Manic Fringe* (Sequence ID NO:3) and Radical Fringe (Sequence ID NO:5) are shown in Tables 1A, 2A and 3A respectively. The corresponding amino acid sequences for Lunatic Fringe protein (Sequence ID NO:2), Manic Fringe protein (Sequence ID NO:4) and Radical Fringe protein (Sequence ID NO:6) are shown in Tables 1B, 2B and 3B respectively.

Undifferentiated mammalian cells appear to express *Lunatic Fringe* but not *Manic Fringe* or *Radical Fringe*. During differentiation, there is a switch over to expression of *Manic* and *Radical* and a cessation of expression of *Lunatic*.

The present invention demonstrates that the three mammalian Fringe proteins may be used to facilitate or block the Notch signal transduction pathway and Notch-dependent processes by regulating the sensitivity of Notch receptors for their specific ligands.

Isolated Nucleic Acids

In accordance with one series of embodiments, this invention provides isolated nucleic acids corresponding to or related to the nucleic acid sequences disclosed herein which encode the murine Fringe proteins, Lunatic Fringe, Radical Fringe and Manic Fringe.

One of ordinary skill in the art is now enabled to identify and to isolate mammalian Fringe genes or cDNAs which are allelic variants of the disclosed Mammalian

Fringe sequences or are homologues thereof, in other species, including humans, using standard hybridization screening or PCR techniques.

In one embodiment, the invention provides cDNA sequences encoding the murine Lunatic Fringe, Manic Fringe and Radical Fringe proteins (Sequence ID NOS: 1, 3 and 5 respectively) comprising the nucleotide sequences of Sequence ID NOS: 2, 4 and 6 respectively.

Also provided are portions of the *Fringe* gene sequences useful as probes in PCR primers or for encoding fragments, functional domains or antigenic determinants of Fringe proteins.

The invention also provides portions of the disclosed nucleic acid sequences comprising about 10 consecutive nucleotides (eg. for use as PCR primers) to nearly the complete disclosed nucleic acid sequences. The invention provides isolated nucleic acid sequences comprising sequences corresponding to at least 10, preferably 15 and more preferably at least 20 consecutive nucleotides of the *Fringe* genes as disclosed or enabled herein or their complements.

In addition, the isolated nucleic acids of the invention include any of the above described nucleotide sequences included in a vector.

Substantially Pure Proteins

In accordance with a further series of embodiments, this invention provides substantially pure mammalian Fringe proteins, fragments of these proteins and fusion proteins including these proteins and fragments.

The proteins, fragments and fusion proteins have utility, as described herein, for the preparation of polyclonal and monoclonal antibodies to mammalian Fringe proteins, for the identification of binding partners of the mammalian Fringe proteins and for diagnostic and therapeutic methods, as described herein. For these uses, the present invention provides substantially pure proteins, polypeptides or derivatives of polypeptides

which comprise portions of the mammalian Fringe amino acid sequences disclosed or enabled herein and which may vary from about 4 to 5 amino acids (e.g. for use as immunogens) to the complete amino acid sequence of the proteins. The invention provides substantially pure proteins or polypeptides comprising sequences corresponding to at least 5, preferably at least 10 and more preferably 50 or 100 consecutive amino acids of the mammalian Fringe proteins disclosed or enabled herein.

The proteins of the invention may be isolated and purified by any conventional method suitable in relation to the properties revealed by the amino acid sequences of these proteins.

Alternatively, cell lines may be produced which overexpress the *Fringe* gene products, allowing purification of the proteins for biochemical characterization, large-scale production, antibody production and patient therapy.

For protein expression, eukaryotic and prokaryotic expression systems may be generated in which a *Fringe* gene sequence is introduced into a plasmid or other vector which is then introduced into living cells. Constructs in which the Fringe cDNA sequence containing the entire open reading frame is inserted in the correct orientation into an expression plasmid may be used for protein expression. Alternatively, portions of the sequence may be inserted. Prokaryotic and eukaryotic expression systems allow various important functional domains of the protein to be recovered as fusion proteins and used for binding, structural and functional studies and also for the generation of appropriate antibodies.

Typical expression vectors contain promoters that direct the synthesis of large amounts of mRNA corresponding to the gene. They may also include sequences allowing for their autonomous replication within the host organism, sequences that encode genetic traits that allow cells containing the vectors to be selected, and sequences that increase the efficiency with

which the mRNA is translated. Stable long-term vectors may be maintained as freely replicating entities by using regulatory elements of viruses. Cell lines may also be produced which have integrated the vector into the genomic DNA and in this manner the gene product is produced on a continuous basis.

Expression of foreign sequences in bacteria such as *E. coli* require the insertion of the sequence into an expression vector, usually a plasmid which contains several elements such as sequences encoding a selectable marker that assures maintenance of the vector in the cell, a controllable transcriptional promoter which upon induction can produce large amounts of mRNA from the cloned gene, translational control sequences and a polylinker to simplify insertion of the gene in the correct orientation within the vector. A relatively simple *E. coli* expression system utilizes the lac promoter and a neighboring lacZ gene which is cut out of the expression vector with restriction enzymes and replaced by the *Fringe* gene sequence. In vitro expression of proteins encoded by cloned DNA is also possible using the T7 late-promoter expression system. Plasmid vectors containing late promoters and the corresponding RNA polymerases from related bacteriophages such as T3, T5 and SP6 may also be used for in vitro production of proteins from cloned DNA. E. coli can also be used for expression by infection with M13 Phage mGPI-2. E. coli vectors can also be used with phage Lambda regulatory sequences, by fusion protein vectors, by maltose-binding protein fusions, and by glutathione-S-transferase fusion proteins.

Eukaryotic expression systems permit appropriate post-translational modifications to expressed proteins. This allows for studies of the *fringe* genes and gene products including determination of proper expression and post-translational modifications for biological activity, identifying regulatory elements in the 5' region of the gene and their role in tissue regulation of protein

expression. It also permits the production of large amounts of normal proteins for isolation and purification, to test the effectiveness of pharmacological agents or as a component of a signal transduction system to study the function of the normal complete protein, specific portions of the protein, or of naturally occurring polymorphisms and artificially produced mutated proteins.

The Fringe DNA sequences can be altered using procedures such as restriction enzyme digestion, DNA polymerase fill-in, exonuclease deletion, terminal deoxynucleotide transferase extension, ligation of synthetic or cloned DNA sequences and site-directed in vitro mutagenesis, including site-directed sequence alteration using specific oligonucleotides together with PCR.

Once the appropriate expression vector containing the selected gene is constructed, it is introduced into an appropriate host cell by transformation techniques including calcium phosphate transfection, DEAE-dextran transfection, electroporation, microinjection, protoplast fusion and liposome-mediated transfection.

The host cell which may be transfected with the vector of this invention may be selected from the group consisting of E. Coli, Pseudomonas, Bacillus subtilis, or other bacilli, other bacteria, yeast, fungi, insect (using baculoviral vectors for expression), mouse or other animal or human tissue cells. Mammalian cells can also be used to express the Fringe proteins using a vaccinia virus expression system.

Methods for producing appropriate vectors, for transforming cells with those vectors and for identifying transformants are described in the scientific literature, for example in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. or latest edition thereof.

The cellular distribution of Fringe proteins in

tissues can be analyzed by reverse transcriptase PCR analysis. Antibodies can also be generated for several applications including both immunocytochemistry and immunofluorescence techniques to visualize the proteins directly in cells and tissues in order to establish the cellular location of the proteins.

The present invention includes effective fragments or analogues of the Fringe proteins described herein. "Effective" fragments or analogues retain the activity of the described Fringe proteins to modulate Notch receptor/Notch ligand interactions.

The term "analogue" extends to any functional and/or chemical equivalent of a mammalian Fringe protein and includes proteins having one or more conservative amino acid substitutions, proteins incorporating unnatural amino acids and proteins having modified side chains.

Examples of side chain modifications contemplated by the present invention include modification of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidation with methylacetimidate; acetylation with acetic anhydride; carbamylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6, trinitrobenzene sulfonic acid (TNBS); alkylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with $NaBH_4$.

The guanidino group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2, 3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation via -acylisourea formation followed by subsequent derivatisation, for example, to a corresponding amide.

Sulfhydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of

mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulfonic acid, phenylmercury chloride, 2-chloromercuric-4-nitrophenol and other mercurials; carbamylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphonyl halides. Tyrosine residues may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodacetic acid derivatives of N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid-, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers or amino acids.

Examples of conservative amino acid substitutions are substitutions within the following five groups of amino acids (amino acids are identified by the conventional single letter code): Group 1: F Y W; Group 2: V L I; Group 3: H K R; Group 4: M S T P A G; Group 5: D E.

Fragments or analogues of the mammalian Fringe proteins of the invention may be conveniently screened for their effectiveness by a variety of methods.

For example, a Drosophila-based assay can be employed. In Drosophila, the mammalian Manic and Radical Fringe proteins interfere with specific Notch-dependent developmental events (eg. Manic Fringe blocks wing margin formation, and causes small eyes and fusion of ocelli in

specific transgenic lines whereas Radical Fringe blocks Margin induction and causes extra bristles to form and can induce wing vein deltas in specific transgenic lines) (Cohen et al. (1997), Nature Genetics, 16, 283-288 and as described herein). Transgenic Drosophila may be used to screen for Fringe proteins, analogues and fragments which enhance or suppress these phenotypes. In addition, drugs which enhance or suppress these phenotypes could be identified which would be useful therapeutically in humans to alter Fringe function and Notch signalling.

Alternatively, a cell culture assay could be used as a screen. It has been reported that differentiation of C2C12 myoblast cells can be blocked in culture by activation of Notch expressed on the cell surface. (Lindsell et al. (1995), Cell, 80, 909-917; Luo et al., (1997), Mol. Cell. Biol., 17, 6057-6067). This activation can occur as a result of presenting DSL ligands to the C2C12 cells. This is achieved by coculturing cells expressing Notch ligands with the C2C12 cells. This assay can be easily adapted to screen for the effect of Fringe proteins, analogues and fragments to regulate the activation of mammalian Notch receptors by their ligands. Similarly, any cell culture system which shows in vitro differentiation dependent on Notch activation may form the basis of a screening assay.

Antibodies

In order to prepare polyclonal antibodies, fusion proteins containing defined portions or all of the Fringe proteins can be synthesized in bacteria by expression of corresponding DNA sequences in a suitable cloning vehicle. Fusion proteins are commonly used as a source of antigen for producing antibodies. Two widely used expression systems for E. coli are glutathione-S-tranferase or maltose binding protein fusions using the pUR series of vectors and trpE fusions using the pATH vectors. The protein can then be purified, coupled to a carrier protein if desired, and mixed with Freund's

adjuvant (to help stimulate the antigenic response of the animal) and injected into rabbits or other appropriate laboratory animals. Alternatively, the protein can be isolated from Fringe protein-expressing cultured cells. Following booster injections at weekly intervals, the rabbits or other laboratory animals are then bled and the sera isolated. The sera can be used directly or purified prior to use by various methods including affinity chromatography employing Protein A-Sepharose, antigen Sepharose or Anti-mouse-Ig-Sepharose. The sera can then be used to probe protein extracts from cells and tissues run on a polyacrylamide gel to identify the Fringe protein. Alternatively, synthetic peptides can be made to the antigenic portions of the proteins and used to inoculate the animals.

The most common practice is to choose a 10 to 15 residue peptide corresponding to the carboxyl or amino terminal sequence of a protein antigen and to chemically cross-link it to a carrier molecule such as keyhole limpet haemocyanin or BSA. However, if an internal sequence peptide is desired, selection of the peptide is based on the use of algorithms that predict potential antigenic sites. These predictive methods are, in turn, based on predictions of hydrophilicity (Kyte and Doolittle (29), Hopp and Woods (30) or secondary structure (Chou and Fasman (31)). The objective is to choose a region of the protein that is either surface exposed such a hydrophilic region or a region conformationally flexible relative to the rest of the structure, such as a loop region or a region predicted to form a β-turn. The selection process is also limited by constraints imposed by the chemistry of the coupling procedures used to attach peptide to carrier protein. A carboxyl-terminal peptide is chosen because they are often more mobile than the rest of the molecule and the peptide can be coupled to a carrier in a straightforward manner using glutaraldehyde. The amino-terminal peptide has the disadvantage that it may be modified post-

translationally by acetylation or by the removal of a leader sequence. A comparison of the protein amino acid sequence between species can yield important information. Those regions with sequence differences are likely to be immunogenic. Synthetic peptides can also be synthesized as immunogens as long as they mimic the native antigen as closely as possible.

It is understood by those skilled in the art that monoclonal anti-Fringe antibodies may also be produced using Fringe protein obtained from cells actively expressing the protein or by isolation from tissues. The cell extracts, or recombinant protein extracts, containing the Fringe protein, are injected in Freund's adjuvant into mice. After being injected 9 times over a three week period, the mice spleens are removed and resuspended in phosphate buffered saline (PBS). The spleen cells serve as a source of lymphocytes, some of which are producing antibody of the appropriate specificity. These are then fused with a permanently growing myeloma partner cell, and the products of the fusion are plated into a number of tissue culture wells in the presence of a selective agent such as HAT. The wells are then screened by ELISA to identify those containing cells making binding antibody. These are then plated and after a period of growth, these wells are again screened to identify antibody-producing cells. Several cloning procedures are carried out until over 90% of the wells contain single clones which are positive for antibody production. From this procedure a stable line of clones which produce the antibody is established. The monoclonal antibody can then be purified by affinity chromatography using Protein A Sepharose, ion-exchange chromatography, as well as variants and combinations of these techniques.

Truncated versions of monoclonal antibodies may also be produced by recombinant techniques in which plasmids are generated which express the desired monoclonal antibody fragment(s) in a suitable host. Antibodies

specific for mutagenic epitopes can also be generated.

The mammalian proteins, Fringe analogues and fragments thereof and/or peptides of the invention are also useful as antigens in immunoassays including enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA) and other non-enzyme linked antibody binding assays or procedures known in the art for the detection of the protein.

Pharmaceutical Compositions

In a further embodiment, this invention provides pharmaceutical compositions for the treatment of mammalian disorders which involve inappropriate Fringe function and/or Notch signalling, comprising a therapeutic amount of a Fringe protein, an analog or an effective derivative thereof in association with a pharmaceutical carrier.

Administration of a therapeutically active amount of a pharmaceutical composition of the present invention means an amount effective, at dosages and for periods of time necessary to achieve the desired result. This may also vary according to factors such as the disease state, age, sex, and weight of the subject, and the ability of the mammalian Fringe protein to elicit a desired response in the subject. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

By pharmaceutically acceptable carrier as used herein is meant one or more compatible solid or liquid delivery systems. Some examples of pharmaceutically acceptable carriers are sugars, starches, cellulose and its derivatives, powdered tragacanth, malt, gelatin, collagen, talc, stearic acids, magnesium stearate, calcium sulfate, vegetable oils, polyols, agar, alginic acids, pyrogen-free water, isotonic saline, phosphate

buffer, and other suitable non-toxic substances used in pharmaceutical formulations. Other excipients such as wetting agents and lubricants, tableting agents, stabilizers, anti-oxidants and preservatives are also contemplated.

The compositions described herein can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable carrier. Suitable carriers and formulations adapted for particular modes of administration are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis the compositions include, albeit not exclusively, solutions of the substance in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The pharmaceutical compositions of the invention may be administered therapeutically by various routes such as by injection or by oral, nasal, buccal, rectal, vaginal, transdermal or ocular routes in a variety of formulations, as is known to those skilled in the art.

Binding partners

The mammalian Fringe proteins, expressed as fusion proteins, can be utilized to identify small peptides that bind to these proteins. In one approach, termed phage display, random peptides (up to 20 amino acids long) are expressed with coat proteins (geneIII or geneVIII) of filamentous phage such that they are expressed on the surface of the phage thus generating a library of phage that express random sequences. A library of these random sequences is then selected by incubating the library with

the mammalian Fringe protein or fragments thereof and phage that bind to the protein are then eluted either by cleavage of Fringe from the support matrix or by elution using an excess concentration of soluble Fringe protein or fragments. The eluted phage are then repropagated and the selection repeated many times to enrich for higher affinity interactions. The random peptides can either be completely random or constrained at certain positions through the introduction of specific residues. After several rounds of selection, the final positive phage are sequenced to determine the sequence of the peptide.

An alternate but related approach uses affinity purification techniques. Fringe proteins are immobilised on a suitable solid support. Preparations such as cell extracts which may contain Fringe protein binding partners are passed over the affinity matrix and any bound material is eluted and microsequenced. Suitable methods are available in the scientific literature, for example in Bartley et al., Nature (1994), 368, 558-560.

Expression cloning, for example through expression of cDNA libraries in Cos or other cells followed by binding of labelled Fringe protein to the transfected cells, may also be used to screen for Fringe protein binding partners, for example as described in Matthews et al., Cell (1991) 65, 973-982.

The identification of proteins or peptides that interact with Fringe Proteins can provide the basis for the design of peptide antagonists or agonists of Fringe protein function. Further, the structure of these peptides determined by standard techniques such as protein NMR or X-ray crystallography can provide the structural basis for the design of small molecule drugs.

Animal Models

The present invention also provides for the production of transgenic non-human animal models for the study of mammalian Fringe gene function, for the screening of candidate pharmaceutical compounds, for the

creation of explanted mammalian cell cultures which express the Fringe proteins or in which a Fringe gene has been inactivated by knock-out deletion, and for the evaluation of potential therapeutic interventions.

The invention enables a transgenic animal, including a transgenic insect, wherein a genome of the animal or of an ancestor of the animal has been modified by introduction of a transgene comprising a mammalian *fringe* gene under the transcriptional control of tissue restricted regulatory elements including the mouse mammary-tumour virus long term repeat sequences.

Transgenic fruit flies which express mammalian Fringe genes may be made as described in the Examples herein. Such transgenic flies may be used to screen for compounds which can repair developmental defects observed in these transgenic flies.

Transgenic animals may also be made and used similarly. Further, transgenic animals with inappropriate expression of Fringe proteins may be examined for phenotypic changes, for example tumour development, and may be used to screen for compounds with potential as pharmaceuticals. Compounds which provide reversal of the phenotypic changes are candidates for development as pharmaceuticals.

Transgenic animal models in accordance with the invention can be created by introducing a DNA sequence encoding a selected mammalian Fringe protein either into embryonic stem (ES) cells of a suitable animal, for example a mouse, by transfection or microinjection, or into a germ line or stem cell by a standard technique of oocyte microinjection.

The ES cells are inserted into a young embryo and this embryo or an injected oocyte are implanted into a pseudo-pregnant foster mother to grow to term.

The techniques for generating transgenic animals are now widely known and are described in detail, for example, in Hogan et al., (1986), and M. Capecchi (1989).

Methods of Treatment

In accordance with one embodiment, the present invention enables a method for preventing or treating a disorder in a mammal characterised by an abnormality in a signal transduction pathway which involves an interaction between a Notch protein and a Notch ligand, by modulating the Notch protein/Notch ligand interaction.

The Notch protein/Notch ligand interaction is modulated, in one embodiment, by administration of a mammalian Fringe protein or an effective fragment or analogue therof.

A further embodiment is a method for treating or preventing such a disorder by promoting or inhibiting the interaction of Notch with its ligands Serrate and Delta by administration of an effective amount of Lunatic Fringe protein, Manic Fringe protein or Radical Fringe protein or of a derivative thereof.

In a further embodiment, the invention enables a method for promoting differentiation of a mammalian cell by suppressing expression of Lunatic Fringe protein in the cell and/or promoting expression of Radical Fringe protein and/or Manic Fringe protein in the cell.

In a further embodiment, the invention enables a method for suppressing differentiation of a cell by suppressing expression of Radical Fringe protein and/or Manic Fringe protein in the cell and/or promoting expression of Lunatic Fringe protein in the cell.

It has been recently demonstrated that the Notch4 receptor is highly expressed in endothelial cells[1]. In addition, the Jagged[1] protein is induced by fibrin in human endothelial cells[2]. Notch signalling may therefore be an important regulator of endothelial cell migration, proliferation and cell fate specification. In humans, vasculature and cardiovascular system malfunction accounts for a very large number of deaths.

One important application of the Fringe proteins, and analogues is regulation of the response of Notch in

mammalian blood vessels. For example, during angioplasty, application of Fringe proteins, Fringe anti-sense oligonucleotides[3] or other reagents to modify fringe function locally may be used to alter Notch activation and therefore the migration and proliferation of cells within vessels. These reagents may also be used to regulate or treat symptoms related to atherosclerosis, cardiovascular disease or diseases related to angiogenesis, including cancer.

Screening Methods

In a further embodiment, the invention enables a method for identifying compounds which can modulate the expression of mammalian a *Fringe* gene comprising

contacting a cell with a candidate compound wherein the cell includes a regulator of a *Fringe* gene operably joined to a coding region; and

detecting a change in expression of the coding region.

In a further embodiment, the invention enables a method for identifying compounds which can selectively bind to a mammalian Fringe protein comprising

providing a preparation including at least one mammalian Fringe protein;

contacting the preparation with a candidate compound; and

determining binding of the Fringe protein to the compound.

Suitable methods for such screening include affinity chromatography, co-immunoprecipitation, biomolecular interaction assay.

In a further embodiment, the invention enables a method for identifying compounds which can modulate the activity of a Fringe protein to promote or inhibit the interaction of a Notch receptor and a Notch ligand.

Methods are also enabled to identify compounds which can modulate the interaction of a Fringe protein with a Notch receptor signal transduction pathway.

As an example, wing development in *Drosophila melanogaster* can be used as a screening tool for evaluating fringe/notch interactions.

Cell culture assays may be developed to measure fringe function *in vitro*. Inhibition of the specific fringe response including an alteration in notch function could be used to assay for chemicals which inhibit or enhance fringe function.

In a further embodiment, the invention enables a method for identifying a compound useful for preventing or treating a disorder in a mammal characterised by an abnormality in a signal transduction pathway which involves an interaction between a Notch receptor and Notch ligand, the method comprising screening candidate compounds for their ability to promote or inhibit the interaction of a Fringe protein with the Notch signal transduction pathway.

In a further embodiment, the invention enables a method for promoting or inhibiting an interaction between a Notch receptor and a Notch ligand comprising administering an effective amount of a Fringe protein or of a fragment, analogue or derivative thereof.

In a further embodiment, the invention enables a method for diagnosing in a subject a disorder characterised by abnormal expression of a Fringe protein comprising

obtaining a tissue sample from the subject;

determining Fringe protein expression in the tissue sample.

Tissue samples could be used for isolation of RNA which would then be subjected to RT-PCR analysis using specific primers for *fringe* genes in order to amplify the cDNA for sequencing. Control tissues could be used for comparison of sequence.

With the identification of the mammalian *Fringe* gene sequences and gene products, nucleotide probes and antibodies raised to the gene products can be used in a variety of hybridisation and immunological assays to

screen for and detect the presence of either a normal or mutated gene or gene product.

Patient therapy through removal or blocking of a mutant gene product, as well as supplementation with a normal gene product by amplification, by genetic and recombinant techniques or by immunotherapy can now be achieved.

Correction or modification of the defective gene product by protein treatment immunotherapy (using antibodies to the defective protein) or knock-out of the mutated gene together with wild-type supplementation is now also possible. Suitable methods are described or referenced for example, in Crystal, R.G. (1995), Science, 270, 404-410.

Fringe proteins as regulators of Notch responsiveness

Three mammalian homologues of *Drosophila fringe* have been isolated. The mammalian proteins share extensive sequence homology with each other as well as with *Xenopus* and *Drosophila* Fringe proteins in the C-terminal region, which is predicted to encode the mature Fringe polypeptide in each case.

Severe loss of function mutants in *Drosophila Fringe* are lethal as homozygotes, and therefore this gene must be essential for development (Irvine and Wieschaus, 1994). *D-fringe* function has thus far only been characterized in wing margin specification (Irvine and Wieschaus, 1994; Kim et al., 1995). D-Fringe is required in dorsal cells and must not be expressed in ventral cells of the wing pouch in order for margin tissue to be induced at the D/V boundary. Destruction of this D/V Fringe+/Fringe- expression boundary through ectopic expression of D-Fringe in ventral cells at the D/V boundary, or through loss of D-Fringe expression in dorsal cells at the D/V boundary both result in loss of margin tissue. The similarity of phenotype caused by ectopic ventral expression and loss of dorsal expression has led to the suggestion that Fringe is a

boundary-organizing molecule (Irvine and Wieschaus, 1994). The presence of a Fringe+/Fringe- expression boundary is therefore thought to be important, rather than simply the presence or absence of Fringe in any particular cell.

Analysis of Fringe function in *Drosophila* wing development must be considered in the context of other molecules which are required for margin induction at the D/V boundary. These include Serrate which is expressed dorsally as well as Delta and Wingless which are expressed ventrally. These four ligands all cooperate to activate Notch exclusively at the D/V boundary. The generation of an ectopic Fringe boundary in the ventral wing pouch must, therefore, be considered in the context of Delta and Wingless which are expressed in the ventral compartment. Similarly, the generation of a novel Fringe boundary in the dorsal wing at the intersection of Fringe$^-$ clones with Fringe expressing dorsal cells must be viewed in the context of the dorsal compartment which expresses Serrate.

Expression of either *Manic Fringe* or *Radical Fringe* in *Drosophila*, using the GAL4^{ptc} driver, results in loss of margin tissue. Disruption of margin formation by these two Fringe proteins is not associated with creation of ectopic margins, indicating that margin destruction and margin induction are genetically separable functions. Other phenotypes induced by these two mammalian fringe genes suggest that *Manic Fringe* and *Radical Fringe* interfere with Notch-mediated processes in several tissues. Loss of function of either the Serrate or the Delta ligands for Notch results in loss of margin formation in the wing (Doherty et al., 1996; Kim et al., 1995), and both Manic and Radical give this same phenotype at the margin. Analysis of margin alone cannot therefore be used to distinguish between activities which directly inhibit Serrate, Delta or Notch. Examination of fly tissues which are dependent on either Serrate or Delta (but not both) indicates that *Manic Fringe* and

Radical Fringe misexpression yielded distinct Serrate-like or Delta-like phenotypes. If these genes inhibited Notch via all Notch ligands, then both genes should give the same phenotypes in each tissue examined.

In every analysis of *Lunatic Fringe* expression during mouse development, it was found that *Lunatic Fringe* was expressed in an undifferentiated cell compartment. Interestingly, in two cases examined in detail, somitogenesis and neurogenesis, it was found that *Lunatic Fringe* expression was localized to cells which were responding to Delta expression in neighbouring cells. It is predicted that Lunatic Fringe may be a co-agonist for Delta by facilitating the Delta-mediated activation of Notch. Separating agonist functions on to two polypeptides, Deltal and Lunatic Fringe, could allow for precise control of Notch activation (Figure 8).

Expression analysis also revealed that *Lunatic Fringe* is shut off as cells differentiate. Differentiation is then accompanied by a Fringe expression switch as *Manic* and/or *Radical Fringe* genes are turned on. This Fringe switch could function to reinforce the differentiation decision and regulate the ratio of undifferentiated cells to their committed progeny.

Fringe regulates Notch activation at boundaries

The data obtained by the inventors on the function of Fringe proteins analyzed in Drosophila and expression in the mouse lead to the model shown in schematic form in Figures 8 and 9. The loss of function *Drosophila fringe* allele fng^{52} produces wing vein deltas (Figure 2B). Ectopic expression in *Drosophila* of *Radical Fringe*, which appears to antagonize some Delta functions, also enhanced the phenotype of fng^{52} /+. This suggests that a rate limiting function of *Drosophila* Fringe may be to facilitate the activation of Notch by Delta. This hypothesis would explain the observation that ectopic Delta can only induce novel wing margins on the dorsal

wing surface (Doherty et al., 1996) which expresses D-Fringe, but not on the ventral wing surface which does not express D-Fringe. In addition, if D-Fringe facilitates the activation of Notch by Delta, then ectopic Fringe would be expected to induce novel ventral margin by cooperating with ventrally expressed Delta to activate Notch. This is also the case, and therefore, all data are consistent with D-Fringe facilitating Delta activation of Notch during wing development.

Such synergy between D-Fringe and Delta to activate Notch, however, does not explain why deletion of Fringe in dorsal clones can induce an ectopic margin (Irvine and Wieschaus, 1994). Perhaps *Drosophila* Fringe, like Manic Fringe, inhibits activation of Notch by Serrate in the wing disc. Such inhibition would explain why ectopic Serrate can only induce novel wing margins on the ventral wing surface which does not normally express D-Fringe (Kim et al., 1995), but not on the dorsal wing surface which does express D-Fringe. In addition, if D-Fringe inhibits the activation of Notch by Serrate, then induction of a novel margin in dorsal tissue would occur at the intersection of *fringe*$^-$ clones with Fringe expressing cells. This induction occurs because dorsal Serrate could activate Notch in the cells of the *fringe*$^-$ clones. Thus a dual mechanism is proposed for regulation of Notch by D-Fringe: (i) Fringe synergizes with Delta to activate Notch at the D/V boundary and (ii) Fringe antagonizes Serrate in the dorsal compartment (Figures 8 and 9).

The question arises as to how dorsally expressed D-Fringe and Serrate and ventrally expressed Wingless and Delta cooperate to induce Notch activation only in a single row of cells on either side of the D/V boundary. It is proposed that Fringe blocks Serrate from functioning everywhere in the dorsal compartment so that the latter can only activate Notch in the ventral cells which abut the dorsal compartment. (Serrate may even

require Wingless to activate Notch in these cells, in which case Wingless and Fringe may both be modifiers of Notch specificity.) In addition, Wingless may block Delta from functioning in the ventral compartment (Axelrod et al., 1996). Delta would only activate Notch in the single row of Fringe-expressing dorsal cells which abut the ventral compartment. Thus, specific co-agonists/antagonists (D-Fringe and perhaps Wingless) are localized in such a way that the membrane bound Notch ligands only activate Notch at the D/V boundary (Figure 9).

Vertebrates may use Lunatic Fringe protein to localize Notch1 activation during somitogenesis, since *Delta1* is highly expressed in the forming somite and *Lunatic Fringe* is highly expressed in the surrounding mesoderm. Delta1 may only activate Notch1 at the boundary of these expression domains. This hypothesis is consistent with the requirement for Notch1 in blocking somite differentiation between the forming somites (Conlon et al., 1995). Similarly, *Lunatic Fringe* expression in the ventricular zone of the neural tube may render cells responsive to Delta1, which inhibits differentiation of ventricular neuroblasts (Chitnis et al., 1995). The three mammalian fringe proteins may be used either to facilitate or to block Notch-dependent processes throughout development and adult life by regulating the sensitivity of Notch for specific membrane-bound ligands.

It is not yet clear from Drosophila studies whether D-Fringe is required for Delta signalling throughout development or just during specific cell fate decisions. It is interesting to note, however, that $GAL4^{ptc}$ driven expression of Radical Fringe inhibited only a small fraction of Delta-dependent processes which occur in tissue where *ptc* is expressed (Muskavitch, 1994). For example, *ptc* is expressed in the eye and yet no phenotype(s) has been observed which would indicate that Delta signalling had been compromised in this tissue of

GAL4^{ptc}/*Radical Fringe* flies. D-Fringe expression has not been detected in the S2 cell line, which when transfected with Notch, can respond to Delta *in vitro* (not shown) (Fortini and Artavanis-Tsakonas, 1994). Therefore Delta does not always require D-Fringe to activate Notch. The two tissues where Radical Fringe inhibited Delta functions, wing and scutellum, are two locations which express Wingless. It is proposed that D-Fringe, and perhaps Lunatic Fringe in mammals, are required coagonists for Delta only in the presence of Wingless or other Wnt family proteins. Somitogenesis and neurogenesis in mammals, like wing margin formation in Drosophila, also require the function of Wnt proteins (Dickinson et al., 1994; Gavin et al., 1990; McMahon et al., 1992; Takada et al., 1994). Fringe proteins may modify the function of membrane-bound Notch ligands only in the presence (for Delta) or absence (for Serrate) of Wnt proteins. Biochemical studies are required to define the precise site of interaction between the Fringes and the Notch receptor system. Candidate interacting proteins through which the Fringe proteins may regulate the sensitivity and specificity of Notch include Notch itself, as well as Delta, Serrate and Wnts.

It is postulated that Lunatic Fringe protein facilitates the local activation of Notch during somitogenesis, neurogenesis and other developmental processes. The mammalian Fringe proteins described herein may potentially be used to block cancer by altering Notch function. They may also be used to regulate skin growth and differentiation when applied topically. It is expected that all developing organ systems will have the potential to respond to these proteins. Any normal process which is regulated by signalling through the Notch receptor may be modulated by administration of the Fringe proteins. Further, any pathological condition or disorder which may be ameliorated by inhibition or promotion of signalling through the Notch receptor may be treated by

administration of the Fringe proteins described herein.

The present invention is not limited to the features of the embodiments described herein, but includes all variations and modifications within the scope of the claims.

EXAMPLES

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of molecular genetics, protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

RT-PCR

Mouse tissues were homogenized in TRIZOL (Gibco BRL), total RNA extracted, and poly(A)$^+$ RNA prepared using Oligotex (Qiagen). mRNA was heated to 95°C for 5 minutes prior to cDNA synthesis and reverse transcription was carried out at 37°C for 1-2 hours in 1x First Strand Buffer (Gibco BRL), 10 mM DTT, 1 mM dNTPs (Pharmacia), 10 U RNasin (Pharmacia), 0.5 mg pd(N)6 (Pharmacia), and 200 U of M-MLV Reverse Transcriptase. cDNA was then used in *Taq* Polymerase PCR reactions containing 1X PCR Buffer (Perkin Elmer), 1 mM $MgCl_2$, 0.2 mM dNTPs, 0.01% gelatin, and 1 mg of forward and reverse primers. Degenerate primers were as follows: Fringe upstream 5' GCC GAA TTC TGG TT(T/C) TG(T/C) CA(T/C) (G/T)TN GA(C/T) GA(C/T) GA(C/T) AA(C/T) TA(C/T)GT (codes for amino acids WFCH(V/F)DDDNYV with 5' EcoRI site); Fringe downstream 5' GCC TCT AGA CA (G/A)AA NCC NGC NCC NCC NGT NGC (G/A)AA CCA (G/A)AA (codes for anti-sense of amino acids FWFATGGAGFC with 5' XbaI site). PCR reaction conditions were as follows: initial denaturation at 96°C for 7 min., followed by 2 cycles of 94°C for 50 s, 50°C for 2 min, 72°C for 2 min, 35 cycles of 94°C for 50 s, 55°C for 2

min, 72°C for 1.5 min, and a final incubation of 72°C for 10 min. PCR products (expected size 216 bp based on the human EST) were run out on 3% Nusieve agarose (Mandel) gels, purified using Qiaex II (Qiagen), digested with EcoRI and XbaI and subcloned into Bluescript (Stratagene) for dideoxy sequencing using Sequenase v2.0 (US Biochemicals). DNA and amino acid sequences were analyzed using MacDNASIS software (Hitachi) and searches for related sequences were done through the BLAST network service (Altschul *et al.*, 1990) provided by the National Center for Biotechnology Information.

Example 1: Isolation of Murine *Fringe* cDNA Clones

Approximately 1 x 10^6 plaques of a mouse embryonic (day 14) cDNA library (Stratagene) were transferred and ultraviolet light cross-linked to uncharged nylon membranes (Qiabrane, Qiagen), and screened with a mixture of ^{32}P-labeled inserts from PCR clones of mouse Lunatic, Manic, and Radical Fringe. Hybridization was performed at 48°C for 24 hours in 1M NaCl, 1% SDS, 10% Dextran Sulphate, 50 mM Tris pH 7.5, 1X Denhardt's, and 100 mg/ml denatured salmon sperm DNA. Filters were washed twice with 2X SSC, 0.5% SDS, once with 1X SSC, 0.5% SDS, and once with 1X SSC, 0.5% SDS. All washes were at 48°C for 30 min. and filters were exposed to Kodak BioMax film for 48 hours. Twenty-two positively hybridizing plaques were identified, purified, and cycle sequencing was performed on 11 excised clones using an ABI Biotechnology Automatic DNA sequencer. Of these 11 mouse clones, 8 were Radical, 1 was Manic, and 2 were Lunatic Fringe. The 5' ends of Lunatic and Manic Fringe were cloned by 5' Race using 5'-AmpliFINDER™ Race Kit (Clontech) following manufacturer's specifications. The 3' specific primer used for Race PCR synthesis of Lunatic Fringe was 5'ATC AGT GAA GAT GAA CGT CAT CTC CTT and the 3' specific primer used for Race PCR synthesis of Manic Fringe was 5'CTG CAG AAC AGT TGG TGA.

The cDNA nucleotide sequences of mouse lunatic fringe, mouse manic fringe and mouse radical fringe are shown in Tables 1A, 2A and 3A respectively and the corresponding predicted amino acid sequences are shown in Tables 1B, 2B and 3B.

Table 4 shows a comparison of the predicted mouse Fringe amino acid sequences (m), with the *Drosophila* fringe (D) (Irvine & Wieschaus, 1994) and *Xenopus* Radical fringe (X) (Wu et al., 1996) amino acid sequences.

Red bar indicates the predicted cleavage site for *Xenopus* and mouse Lunatic Fringe proteins. The blue arrows correspond to the amino acid sequences on which degenerate oligonuceotide primers were designed for RT-PCR cloning of the mammalian *Fringe* gene family and red asterisk denotes conserved cysteine residues. Identical residues are boxed in yellow highlight.

Mammalian Fringe family

The public data bases were searched for mammalian (human and mouse) sequences with homology to the *Drosophila fringe* gene. One such sequence, which had been obtained from a three month human brain cDNA library, was identified in the expressed sequence tag database (Accession number F13368). Comparison of the potential translated products from this EST with *Drosophila* Fringe revealed that one reading frame encoded two stretches of almost perfect match with D-Fringe (12 of 13 amino acids and 11 of 11 amino acids respectively). Degenerate oligonucleotide primers to these two regions were designed (Table 4) and PCR was performed with cDNA from several developing mouse tissues. PCR products were cloned, sequenced and found to contain a mixture of sequences from three genes, including a mouse orthologue of the human EST noted above. A mouse embryo cDNA library was then screened with these three PCR derived probes to isolate the corresponding full length cDNA clone for each gene. These three genes have been named *Lunatic fringe*, *Manic fringe* and *Radical fringe* (the original EST was a fragment of human *Radical fringe*).

Multiple overlapping clones were isolated for *Radical Fringe* and the sequence of the full coding region obtained from these cDNAs. This sequence is shown in Table 3A and its predicted amino acid sequence in Table 3B. In contrast, the 5' ends of *Lunatic* and *Manic fringe* genes were not obtained in this screen. 5' Rapid Amplification of cDNA Ends, or 5' RACE, was used on mouse brain cDNA to obtain the 5' regions of both genes. The full coding regions for *Lunatic fringe* and *Manic fringe* were derived from overlapping sequences obtained from 5'RACE clones and the cDNA clones isolated above. The full coding regions of *Lunatic fringe* and *Manic fringe* are shown in Tables 1A and 2A respectively, with their predicted amino acid sequences in Table 1B and 2B.

Analysis of the predicted amino acid sequence of the three murine *fringe* genes reveals that in each case an

N-terminal signal sequence is present to target these proteins to the secretory pathway (Table 4). The red bar in Table 4 indicates the predicted cleavage site for *Xenopus* and mouse lunatic fringe proteins (Wu et al., 1996). The N-terminus of each protein is variable in both length and sequence. Notably, the *Drosophila* Fringe protein N-terminus is significantly longer than all vertebrate Fringe proteins (Irvine and Wieschaus, 1994; Wu et al., 1996). In contrast, the C-terminal 270 amino acids of all Fringe proteins are very highly conserved (starting at residue 152 in Table 4 multiple sequence alignment). In this region, mouse and *Xenopus* Lunatic Fringe proteins are 77% identical; mouse and *Xenopus* Radical Fringe proteins are 59% identical; mouse Lunatic, Manic and Radical Fringe proteins are greater than 50% identical to each other and all vertebrate Fringes are approximately 30% identical to the fruit fly protein (Irvine and Wieschaus, 1994; Wu et al., 1996). Drosophila *fringe* encodes seven cysteine residues which are thought to form disulfide bonds in the native protein (Irvine and Wieschaus, 1994). All vertebrate Fringe proteins (including the three described herein and the two Xenopus published fringe proteins (lunatic, radical) (Wu et al., 1996) contain six of these cysteines at identical positions suggesting that they may form an essential scaffold for this protein family. In addition, the spacing of all conserved residues in the Fringe C-terminal region is nearly identical, with only two single amino acid gaps being necessary to line up all vertebrate proteins with each other and with the *Drosophila* protein.

The *Xenopus Lunatic fringe* gene contains a poorly conserved N-terminal region between the leader peptide and a basic motif which is predicted to be the target of proteolytic processing required for maturation of a functional ligand (Wu et al., 1996). The mouse *Lunatic fringe* gene also encodes a poorly conserved N-terminal putative "pro region" followed by a basic motif, and

therefore, is likely produced as an inactive precursor. In contrast, the Manic and Radical Fringe predicted proteins contain only a few amino acid residues between the leader sequence and the conserved C-terminal region common to all Fringe proteins. Manic Fringe does not contain a basic cleavage sequence and encodes only 29 amino acids from the start codon to the region where Lunatic Fringe is predicted to be cleaved. These 29 amino acids code for little more than the leader sequence, thus Manic Fringe may be secreted in an active form which does not require proteolytic cleavage. The mouse Radical Fringe protein also lacks a tetrabasic cleavage site and contains a shorter N-terminus than the *Xenopus Radical Fringe* gene. From start codon to the location of predicted cleavage in *Lunatic fringe* genes, the mouse *Radical fringe* cDNA only encodes forty four amino acids including the leader sequence (*Xenopus Radical fringe* encodes seventy one amino acids in the corresponding region). Like mouse Manic Fringe, mouse Radical Fringe may not require regulated proteolytic activation.

Example 2: Expression of Fringe in mice

(a) Northern Blot Analysis

Total RNA from adult mouse brain, thymus, heart, lung, liver, kidney, spleen, skeletal muscle, and ES cells was prepared using Trizol (Gibco, BRL). RNA samples (10 ug) were electrophoretically separated on a 1.2% agarose/formaldehyde gel, transferred and ultraviolet light cross-linked to Genescreen (Dupont). Hybridization was performed at 65°C in 1M NaCl, 10% Dextran Sulphate, 1% SDS and 100 mg/ml denatured salmon sperm DNA. Blots were washed twice for 5 min. at RT in 2X SSC, 0.1% SDS, twice for 5 min. at RT in 0.2X SSC, 0.1% SDS, twice for 15 min. at 42°C in 0.2X SSC, 0.1% SDS, and twice for 15 min. at 68°C in 0.1X SSC, 0.1% SDS. Blots were exposed for 2 - 4 days to Kodak BioMax film in

the presence of an intensifying screen. A mouse embryo multiple tissue northern blot (Clontech) was probed using the manufacturer's specifications. The probe for Manic Fringe was a 159 bp EcoRI-PvuII fragment which starts 383 bp downstream of the last amino acid in the coding sequence. The probe for Lunatic Fringe was 2kb EcoRI insert from pBK-phagemid vector (clone 24), and the probe for Radical Fringe was a 1.5kb EcoRI insert from pBK-phagemid vector (clone 89). All probes were random primed-labeled with [a-^{32}P]dCTP and 2 x 10^6 cpm/ml were used for hybridization.

The results are shown in Figures 3 and 4.

(b) Tissue section in situ hybridization

In situ hybridization experiments were performed using 8-μ paraffin or frozen sections from developmentally-staged C.B.-17 mouse embryos. Midday of the time of appearance of vaginal plugs was considered as 0.5 dpc to time pregnancies. For paraffin sections, embryos were fixed overnight in 4% paraformaldehyde, dehydrated in ethanol and embedded in paraffin. For cryosections, embryos were protected by embedding in OCT compound (Miles) prior to freezing in liquid N_2. Pretreatments of frozen sections included fixing in 4% paraformaldehyde for 1 h., followed by proteinase K digestion (20 mg/ml, 7.5 min, 25°C) and acetylation (0.1 M triethanolamine pH 8.0, 0.25% acetic anhydride, 10 min, 25°C). Subsequently, the sections were dehydrated with ethanol and air-dried prior to addition of hybridization solution.

Riboprobes were synthesized using T7 RNA polymerase (Pharmacia, Boehringer Mannheim), T3 RNA polymerase (Pharmacia), and SP6 RNA polymerase (Pharmacia) according to the protocol of the manufacturer.

pBK-*Radical*dKpnI(clone 16) was used to synthesize sense (T3) and antisense (T7) probes of 709 bp which span from nt 418 of *Radical* fringe to 118 nt downstream of coding

sequence. A probe for Lunatic Fringe was generated by PCR using the following primers: 5' GAATTC CTG CTG TTC GAG ACC TGG ATC (contains EcoRI site) and 5' AGATCT ACC AGG ATT GTA GAA GAT CGC (contains BglII site) and pBK-*Lunatic* (clone 24) as template. The 756 bp PCR product which spans nt 273 to nt 1030 of the *Lunatic* coding sequence was subcloned into pGemT (Promega) and sense and antisense riboprobes synthesized with SP6 and T7 polymerase respectively. pBK-*Manic* (clone 30) was digested with EcoRI and a 426 bp EcoRI fragment from 3' untranslated region of *Manic* fringe (begins 284 bp downstream of last coding nt) was subcloned into phosphatase-treated EcoRI-digested pBluescript vector (Stratagene). Sense and antisense *Manic* riboprobes were synthesized from this plasmid using T3 and T7 polymerases respectively. A probe for mouse Serrate-1 was generated using the following primers: 5' TCC AGC TGA CAG AGG TTT CC and 5' GAC CAG AAT GGC AAC AAA ACC TGC. The 937 bp PCR product, which covers nt 641-1578 of the rat sequence was designed by searching for stretches of DNA identity between rat and chicken *Serrate-1*, which was predicted to be identical in mouse *Serrate-1*. This PCR product was subcloned into pGemT (Promega) and antisense riboprobes generated by transcribing with T7 RNA polymerase. A 777 bp ScaI/PstI fragment of mouse Delta spanning nt 669-1446 was subcloned in pBK (Stratagene) and antisense riboprobes generated using T3 RNA polymerase.

Pretreatment of paraffin sections and hybridization to [a^{33}P]UTP-labeled sense and antisense probes (15,000-40,000 cpm/ml) were conducted as described by Hui and Joyner (Hui and Joyner, 1993), with the following modifications. The hybridization and washing steps omitted use of DTT. Following RNase treatment, the sections were washed sequentially with 2x SSC, 1x SSC and 0.5x SSC at 37°C, 10 min each, and with 0.1x SSC at 65°C for 30 min. Exposure of slides to emulsion was allowed to proceed for 1-3 weeks and, after development, the tissues were stained lightly with hematoxylin and eosin.

(c) Whole Mount In Situ Hybridization

Embryos were dissected into PBS and extraembryonic tissues removed. Embryos were fixed overnight at 4°C with 4% paraformaldehyde (PFA) in PBS, rinsed once with cold PBT (PBS with 0.1% Tween 20) and dehydrated through an ascending methanol series (25%, 50%, 75%) in PBT and then stored in 100% methanol at -20C until further use. Antisense riboprobes were synthesized from the same DNA templates as described previously for section *in situ.*, using a digoxygenin RNA labeling kit (Boehringer Mannheim). Embryos were rehydrated through a descending methanol series rinsed twice in PBT, and then bleached for 1 hour at RT in 6% hydrogen peroxide in PBT. After three rinses with PBT, embryos were permeabilized with 10 ug/ml proteinase K (5 min. for E9.5 embryo and 2 min. for E8.5 embryo), rinsed twice with PBT and then fixed with Glutaraldehyde 0.2%/PFA 4%/PBT for 20 min at RT. After fixation, embryos were washed 4X with PBT, washed once with hybridization buffer (50% formamide, 5X SSC [pH 4.5], 50 ug/ml yeast tRNA, 1% SDS, 50 ug/ml heparin), and incubated with 1.5 ml of fresh hybridization buffer for 1 hr at 70°C. Digoxygenin-labeled riboprobe (1.5 mg) was added directly and embryos were incubated overnight at 70°C.

Following hybridization, embryos were washed twice for 30 min at 70°C with solution 1 (50% formamide, 5X SSC [pH 4.5], 1% SDS), washed once for 10 min at 70°C with 50/50 solution 1/solution 2 (0.5 M NaCl, 0.01 M Tris [pH 7.5], 0.1% Tween-20), rinsed 3X with solution 2 at RT, rinsed once at RT with solution 3 (50% formamide, 2X SSC [pH 4.5]), and twice for 30 min at 65°C with solution 3. Embryos were then rinsed 3X at RT with TBS-TL (137 mM NaCl, 2.7 mM KCl, 25 mM Tris [pH 7.5] plus 2 mM Levamisole and 0.1% Tween 20) and then incubated for 1 hr at RT with TBS-TL containing 10% heat-inactivated (65C for 30 min) goat serum to prevent non-specific binding of

antibody. Anti-digoxygenin Fab alkaline phosphatase conjugate (1/5000, Boehringer Mannheim) was preabsorbed in TBS-TL with 1% heat-inactivated goat serum and approximately 3 mg heat-inactivated embryo powder per ml antibody. After an overnight incubation at 4°C with the preabsorbed antibody, embryos were rinsed 3X with TBS-TL, washed 4X for 1 h with TBS-TL at RT, and then left overnight at 4°C in fresh TBS-TL. The buffer was exchanged by washing 3X for 10 min with NTMT (0.1 M NaCl, 0.1 M Tris [pH 9.5], 0.05 M $MgCl_2$, 0.1% Tween-20, 2mM levamisole), and the antibody detection reaction was performed by incubating embryos with detection solution (NTMT with 0.25 mg/ml nitroblue tetrazolium and 0.13 mg/ml 5-bromo-4-chloro-3-indolulphosphate toluidinium). Detection reactions were complete within 15 min - 1 hour and then embryos were washed twice in PBT. Color was intensified by dehydration/rehydration through ascending and descending methanol/PBT rinses. Embryos were then cleared through 50% and 80% glycerol in CMFET (137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5mM $KH2PO_4$, 0.7 mM EDTA, 0.1% EDTA, 0.1% Tween-20) and whole embryos were photographed under transmitted light using a Leica MZ12 microscope with Kodak Tungsten 160 ASA film.

Results of the *in situ* hybridisation studies are shown in Figures 5 to 7

Mammalian Fringe gene expression in development

To identify tissues which express mammalian *fringe* homologues, Northern blot analysis was performed on RNA derived from mouse embryos and adult tissues. The three genes were expressed at all stages of mouse embryonic development analyzed, from day seven of gestation to day seventeen (Figure 3). Two transcripts were detected for Manic and Radical *Fringe* genes in mouse embryos. In addition, the three *fringe* genes were widely expressed in adult tissues, with *Lunatic fringe* having a more restricted expression pattern than either *Manic* or *Radical* (Figure 4). Liver RNA samples were underloaded

and each of the three *fringe* genes can be detected in this tissue on longer exposures of these northern blots.

In situ hybridization analysis revealed that in many cases the expression of *fringe* genes in both embryos and adults was localized to tissues undergoing development or differentiation. *Lunatic Fringe* is highly expressed during neurogenesis and somitogenesis. Mouse embryos at 8.5 and 9.5 days of gestation (E8.5 and E9.5) express *Lunatic Fringe* in two stripes which surround the forming somite (Figure 5A and 5B). These two stripes move with time towards the posterior of the embryo as new somites are generated, suggesting that the *Lunatic Fringe* gene is involved in the segmentation of mesoderm into somites. In addition, *Lunatic Fringe* is expressed throughout the developing central nervous system in the undifferentiated neuroblast layers of the neural tube, brain, and otic vesicle. This expression continues in uncommitted neuroblasts as mice continue to develop (Figure 5C, 5E and data not shown). In day 11.5 embryos (Figure 5C), *Lunatic Fringe* is also expressed in the myotome which contains undifferentiated myoblasts and in the intervertebral mesenchyme (data not shown). Throughout development, this *fringe* gene continues to be expressed in proliferating cells in the ventricular zones of the nervous system, in uncommitted neuroblasts in the retina (C.C. and B.G. unpublished) and in the perichondrium which contains proliferating chondroblasts (Figure 5D and data not shown). *Lunatic Fringe* is also expressed in developing organs and continuously developing systems in adult mice. For example, it is expressed in the fetal heart and hematopoetic cells in the fetal liver at E12.5 (not shown), in S-shaped bodies of the developing kidney (Figure 5G), in the thymic medulla (Figure 5H), in a subset of splenic lymphocytes (Figure 5J), in the basal epithelium of skin (not shown) and similarly in the basal epithelium of the tongue (Figure 6E). It is concluded that Lunatic Fringe is expressed in cells which have yet

to complete their developmental program and remain competent both to proliferate and to differentiate. The fact that lunatic fringe is expressed in the basal epithelium of the skin which is continuously undergoing cellular regeneration and differentiation suggests that this protein could be applied topically to the skin as a therapeutic agent to alter abnormal skin growth seen in various skin diseases.

Manic Fringe and Radical Fringe, on the other hand, are often expressed in cells of a more committed cell fate. Both of these genes are expressed in the marginal zones of the neural tube and brain throughout development (Figure 5F and data not shown). Both Manic and Radical Fringe are expressed from day E11.5 to E13.5 in the dorsal root ganglia. These genes are also expressed in E12.5 fetal liver, and in the suprabasal epithelium of both tongue and skin. Manic Fringe also appears to be very highly expressed in megakaryocytes present in the adult spleen (Figure 5K and 5L).

Differentiation induced Fringe gene switch

In many developing tissues, as described above, the "stem cell" population which is undifferentiated and proliferating expressed high levels of *Lunatic Fringe*. In contrast, *Manic* and *Radical Fringe* genes do not seem to be expressed in uncommitted cell compartments. Sections of two tissues where stem cells and their differentiated progeny are physically separated have been analysed. Sections through the neural tube in day 10.5 embryos reveal that *Lunatic Fringe* is expressed in the ventricular zone which corresponds to the neuroblastic population but not in the marginal zone, which contains differentiated neurons (Figure 6A). Adjacent sections probed with *Manic* or *Radical Fringe* riboprobes reveal a striking complementary expression profile for these two genes. As neurons are born, they turn off *Lunatic - Fringe*, leave the ventricular zone and turn on both *Manic* and *Radical Fringe* genes (Figure 6B and 6C). Similarly,

tongue epithelium is continuously regenerated through division of basally located stem cells which express *Lunatic Fringe* (Figure 6D). As cells differentiate, they move apically, turn off *Lunatic Fringe* and turn on both *Manic* and *Radical Fringe* genes (Figure 6E and 6F).

Lunatic Fringe and Deltal expression domains intersect

The process of differentiation is often regulated by Notch and its ligands (Artavanis-Tsakonas et al., 1995). The importance of Notch in differentiation and development of both somites and neural tube has been demonstrated genetically in vertebrates (Chitnis et al., 1995; Chitnis and Kintner, 1996; Coffman et al., 1993; Conlon et al., 1995; Swiatek et al., 1994). During somitogenesis, Notch1 is required for proper segmentation of presomitic mesoderm into somites (Conlon et al., 1995). Notch1 is not, however, required to form somite-like tissue (Conlon et al., 1995). This observation suggests that activation of Notch may block differentiation of mesoderm into somite tissue at the boundary between adjacent somites. Delta family Notch ligands are typically turned on as cells commit to differentiate (Muskavitch, 1994). Analysis of mouse Deltal expression in mesoderm undergoing somitogenesis in E8.5 embryos reveals that Deltal is most strongly expressed in the forming somite (Figure 7A) (Bettenhausen et al., 1995). *Lunatic Fringe* is expressed at this stage in two bands which surround the forming somite (Figure 7B). Mouse *Serrate1/Jagged* is also weakly expressed in two bands which surround the forming somite (Figure 7C). Lunatic Fringe may control the sensitivity or selectivity of Notch for its ligands, perhaps by ensuring that Deltal and Serrate1 only activate Notch1 in cells between the forming somites.

In contrast to the developing somites, *Deltal* and *Lunatic Fringe* genes are expressed in overlapping domains within the developing neural tube (Figure 7D and 7E). Deltal is turned on as cells differentiate into the three

major types of neurons (sensory neurons, interneurons and motor neurons) (Chitnis et al., 1995; Henrique et al., 1995) and activates Notch1 in the remaining neuroblastic layer to prevent differentiation. *Serrate1/Jagged* is expressed in complementary stripes in the neural tube which express neither *Delta1* nor *Lunatic Fringe* (Figure 7F) (Lindsell et al., 1995; Myat et al., 1996). The function of Serrate1 in regulating neural tube development is unknown, although by analogy to the role of Drosophila Serrate in imaginal disc proliferation (Speicher et al., 1994), this mammalian *Serrate* gene may regulate proliferation rather than differentiation of neuroblasts. Thus, in two tissues which are known to undergo Notch dependent patterning (somites and neural tube), *Lunatic Fringe* appears to be expressed in cells which are responding to Delta.

Example 3: Ectopic Expression of Mouse Fringe in Drosophila

An EcoRI fragment containing the entire mouse *Radical* fringe open reading frame was purified from pBK-phagemid vector (clone 89) and ligated with phosphatase-treated EcoRI digested transformation vector pUAST, which contains several GAL4 upstream activator sequences and a minimal promoter (Brand and Perrimon, 1993). The 5' end of Manic Fringe was PCR-modified to contain Kozak consensus sequence (5' GAT CTA CCA ATG G) and an ApaI site was introduced by PCR at nt 304-309 to allow ligation with pBK-phagemid vector *Manic* fringe cDNA (clone 8). The entire *Manic* fringe cDNA was then subcloned as a BglII fragment into phosphatase-treated BglII-digested transformation vector pUAST (Brand and Perrimon, *supra*). The recombinant plasmids, pUAST-*Radical* and pUAST-*Manic*, with the open reading frames in the correct orientation relative to the promoter, were used to transform *Drosophila* embryos using standard microinjection procedures (Spradling, 1986).

For analysis of ectopic expression, transgenic flies carrying pUAST-*Manic* and pUAST-*Radical* were crossed to GAL4 enhancer trap lines. The GAL4 drivers used were $GAL4^{ptc}$ (Hinz et al., 1994), $GAL4^{C5}$ which is expressed throughout the wing disc pouch (Yeh et al., 1995), and GAL4C96 which is expressed only along the D/V boundary (Gustafson and Boulianne, 1996). These crosses were repeated with several independent transgenic lines for pUAST-*Manic* and pUAST-*Radical*. Progeny of such crosses were scored for defects. In this way the mammalian *fringe* genes were expressed in cells where the *patched* gene is expressed (Hinz et al., 1994) which includes specific locations in the eye, wing, ocelli, and most if not all other imaginal discs. In the wing imaginal disc, *patched* is expressed in a stripe of cells on the anterior side of the A/P boundary (Hinz et al., 1994; Kim et al., 1995). Wings were dissected from adult flies, mounted in GMM (Lawrence et al., 1986) and photographed using a Zeiss Axioskop. Pictures of adult fly eyes were obtained by Scanning Electron Microscopy using standard proceedures (Tomlinson and Ready, 1987).

Results are shown in Figures 2 and 3.

Loss of endogenous wing margin

Ectopic expression of *D-fringe* using the $GAL4^{ptc}$ line leads to a loss of wing margin tissue at the A/P boundary. This phenotype is believed to occur because ectopic expression of D-Fringe in ventral cells destroys the natural D/V Fringe boundary at this location (Kim et al., 1995). In addition, expression of *D-fringe* in the ventral compartment causes the creation of a new Fringe boundary in this compartment, and therefore, an ectopic wing margin is generated on the ventral surface of the wing (Kim et al., 1995). In contrast, expression of either Manic or Radical Fringe causes the loss of endogenous margin tissue without the generation of an ectopic margin on the ventral surface (Figure 1B and 1C). Notably the loss of wing margin is more dramatic in

Manic Fringe-expressing flies than in *Radical Fringe*-expressing flies. Radical Fringe usually only induces loss of margin tissue when expressed at high levels either by an extra copy of the $GAL4^{ptc}$ driver, extra copies of the pUAST transgene or when present in sensitized genetic backgrounds (see below).

These results demonstrate that the loss of wing margin tissue induced by ectopically expressed *fringe* genes is a genetically distinct function from the creation of novel ectopic margins. Loss of margin tissue is normally associated with a loss of Notch activation at the D/V boundary (Couso and Martinez Arias, 1994). Induction of novel margins is associated with inappropriate activation of Notch (Doherty et al., 1996; Kim et al., 1995; Rulifson and Blair, 1995). $GAL4^{ptc}$-driven expression of either of these mammalian fringes, like *D-fringe*, causes the disruption of normal margin; but unlike *D-fringe*, they do not encode the function(s) necessary for induction of an ectopic ventral margin. Manic Fringe and Radical Fringe, therefore, appear to inhibit Notch activation by its ligands, Serrate and/or Delta, at the D/V boundary, but seem unable to induce Notch activation in either the dorsal or ventral compartments. These two mammalian Fringes do not mimic Drosophila Fringe because they fail to induce a new margin in the ventral compartment, and do not inhibit Drosophila Fringe; loss of Drosophila Fringe function in the dorsal compartment induces an ectopic dorsal margin (Irvine and Wieschaus, 1994).

Manic and Radical Fringe proteins inhibit distinct Notch ligand dependent processes.

$GAL4^{ptc}$-driven expression of *Manic* and *Radical fringe* genes induces phenotypic effects in other tissues. Manic Fringe induces a dramatic reduction in size of the eye (Figure 1E as compared to 1D and 1F) and fused ocelli (not shown). Wing scalloping (loss of margin) and dramatic reduction of eye size are both phenotypes

associated with loss of Serrate function (Speicher et al., 1994). *Radical Fringe* flies on the other hand had normal eyes but an extra pair of scutellar setae within the normal proneural region (not shown) (Simpson, 1996). Extramacrochaetae/setae within the proneural region and wing scalloping both represent a failure of processes which depend on Delta signalling (Artavanis-Tsakonas et al., 1995; Muskavitch, 1994).

In order to characterize in greater detail the effect of Manic Fringe and Radical Fringe on wing development, the UAS mammalian-Fringe transgenic lines were crossed to other enhancer trap lines which express GAL4 in distinct wing compartments. The $GAL4^{C96}$ line expresses GAL4 at the D/V boundary in the future wing margin (Gustafson and Boulianne, 1996). Crossing Manic Fringe lines to $GAL4^{C96}$ lines produced a dramatic loss of margin tissue and reduction of wing size (Figure 1G). In contrast, crosses between Radical Fringe lines and $GAL4^{C96}$ produced a small loss of margin tissue in some but not most flies (not shown). The $GAL4^{C5}$ enhancer trap line expresses GAL4 in all cells which will become the wing blade (Yeh et al., 1995). Crosses between this line and *Manic Fringe* flies also produced a dramatic loss of margin and wing blade tissue (Figure 1H). In contrast, crosses between $GAL4^{C5}$ and the *Radical Fringe* flies produced wings with small vein deltas, or vein splitting (Figure 1I, see insert). These distinct phenotypes are characteristic of loss of Serrate function in the case of Manic Fringe, and loss of Delta function in Radical Fringe flies. Expression of either mammalian *fringe* gene at the D/V boundary (as in ptcGAL or $GAL4^{C96}$ crosses) may induce a similar wing phenotype because both Serrate and Delta are required for induction of normal margin tissue and wing growth. Thus, examination of Manic and Radical Fringe effects in other tissues of ptcGAL crosses (eyes and bristles) and in other regions of the wing (as in $GAL4^{C5}$ crosses) has identified distinct properties of these two mammalian Fringe proteins.

Additional crosses were performed to test for genetic interactions between ectopically expressed *Radical Fringe* and the endogenous Drosophila *fringe* gene as well as with other signalling molecules involved in wing margin induction at the D/V boundary. As mentioned above, GAL4^{ptc}/UAS-*Radical Fringe* did not produce a phenotype when both GAL4^{ptc} and *Radical Fringe* were present at single dose (Figure 2A). The Drosophila *fringe* hypomorphic allele *fng*^{52} shows a weak wing vein splitting or "delta" phenotype and normal margin in heterozygotes (Figure 3B) (Irvine and Wieschaus, 1994). When GAL4^{ptc}/UAS-*Radical Fringe* flies were also heterozygous for the *fng*^{52} *D-Fringe* allele, significant wing scalloping was observed (Figure 2C). This genetic interaction between *Radical Fringe* and *D-Fringe* suggests that Radical Fringe interferes with an essential function of D-Fringe. The mild wing vein deltas observed in *fng*^{52} flies suggests that D-Fringe is required for Delta to stimulate Notch. Indeed, the wing vein deltas observed in GAL4^{C5}/UAS-*Radical Fringe* flies, described above, indicates that Radical Fringe interferes with Delta stimulation of Notch.

It has been demonstrated previously that both Delta and Serrate function through Notch to induce margin tissue on either side of the D/V boundary. Loss of either of these Notch ligands or of Notch itself results in a loss of margin (Doherty et al., 1996; Kim et al., 1995). In addition, ventral expression of the secreted protein Wingless is required early in wing development for margin induction (Couso and Martinez Arias, 1994). Flies heterozygous for a loss of function *Delta* allele have widened wing veins as well as "deltas" where the veins intersect the wing margin (Figure 2D) (Doherty et al., 1996; Muskavitch, 1994). The margin in these flies however is normal. Flies which are heterozygous for *delta* and also express GAL4^{ptc}/UAS-*Radical Fringe* show a loss of margin tissue (Figure 2H). Expression of the GAL4^{ptc}/UAS-*Radical Fringe* transgene combination also

enhances or reveals a phenotype in combination with heterozygous *Serrate*, *Notch* and *wingless* loss of function alleles (Figure 2F through 2K).

Thus, Radical Fringe inhibits the normal margin specification which depends on D-Fringe, Delta, Serrate, Wingless and the Notch receptor. These dosage sensitive interactions may be a result of all four ligands functioning together to activate Notch only at the D/V boundary. *Radical Fringe* may, therefore, interfere with wing margin induction by inhibiting only one of the four essential ligands, Delta. It remains formally possible that Drosophila and mouse *Fringe* genes studied here function through some novel receptor and signal transduction system, however, the simplest interpretation of our data involve a very proximal regulation of Notch-ligand function by the Fringe proteins.

Example 4:

Dimerisation of Fringe Proteins: Generation of Dominant Inhibitory mutants:

Murine Lunatic fringe, Manic Fringe and Radical Fringe were each tagged using PCR-mediated mutagenesis on their C-termini with the Flag epitope. These chimeric cDNAs were then cloned into the eukaryotic expression vector pCDNA3. Each Fringe expression construct was transfected into Cos cells and lysates were analyzed for production of tagged Fringe protein by Western blot with anti-Flag antibodies. Independently, we tagged the three mammalian fringe genes with C-terminal myc-epitope tags in the pCDNA3 vector. These proteins were expressed in transfected Cos cells and the myc-tagged Fringe proteins detected on Western blots from cell lysates using anti-myc antibodies.

In order to test for dimerization of the Fringe proteins, we transfected Flag-epitope tagged Lunatic Fringe with myc-tagged Lunatic Fringe, myc-tagged Manic Fringe or myc-tagged Radical Fringe. Cell lysates were prepared and immunoprecipitated with anti Flag

antibodies. In each case, precipitated Flag-tagged Lunatic Fringe protein coprecipitated the myc-tagged Fringe protein as detected on western blot analysis using anti-myc antibodies. Similarly, Flag-tagged Manic Fringe could associate with myc-tagged Lunatic, Manic and Radical Fringe in cells. Finally the Flag-tagged Radical Fringe protein also dimerized with myc-tagged Lunatic, Manic and Radical Fringes indicating that all dimeric combinations of the three Fringe polypeptides are capable of forming in cells.

These dimeric interactions suggest that six distinct Fringe dimeric complexes exist in vivo. In addition, this result suggests that the Fringe proteins may function as dimers to regulate the sensitivity of Notch Receptors for their ligands. The dimeric nature of the Fringes can be used to identify or generate dominant inhibitory alleles or mutants of each Fringe. It is expected that mutant Fringes can be made which can either; (i) still dimerize with wild type proteins but cannot form productive interactions with other Fringe partners or (ii) still interact with Fringe-binding partners but are unable to dimerize with wild type Fringe proteins. Such mutants can be used to block endogenous Fringe function.

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TABLE 1A

ATGCTCCAGCGGTGCGGCCGGCGCCTGCTGCTGGCGCTGGTGGGCGCGCTGTTGGCT
TGTCTCCTGGTGCTCACGGCCGACCCGCCACCGACTCCGATGCCCGCTGAGCGCGGA
CGGCGCGCGCTGCGTAGCCTGGCGGGCTCCTCTGGAGGAGCTCCGGCTTCAGGGTCC
AGGGCGGCTGTGGATCCCGGAGTCCTCACCCGCGAGGTGCATAGCCTCTCCGAGTAC
TTCAGTCTACTCACCCGCGCGCGCAGAGACGCGGATCCACCGCCCGGGGTCGCTTCT
CGCCAGGGCGACGGCCATCCGCGTCCCCCCGCCGAAGTTCTGTCCCCTCGCGACGTC
TTCATCGCCGTCAAGACCACCAGAAAGTTTCACCGCGCGCGGCTCGATCTGCTGTTC
GAGACCTGGATCTCGCGCCACAAGGAGATGACGTTCATCTTCACTGATGGGGAGGAC
GAAGCTCTGGCCAAGCTCACAGGCAATGTGGTGCTCACCAACTGCTCCTCGGCCCAC
AGCCGCCAGGCTCTGTCCTGCAAGATGGCTGTGGAGTATGACCGATTCATTGAGTCT
GGGAAGAAGTGGTTCTGCCACGTGGATGATGACAACTACGTCAACCTCCGGGCGCTG
CTGCGGCTCCTGGCCAGCTATCCCCACACCCAAGACGTGTACATCGGCAAGCCCAGC
CTGGACAGGCCCATCCAGGCCACAGAACGGATCAGCGAGCACAAAGTGAGACCTGTC
CACTTTTGGTTTGCCACCGGAGGAGCTGGCTTCTGCATCAGCCGAGGGCTGGCCCTA
AAGATGGGCCCATGGGCCAGTGGAGGACACTTCATGAGCACGGCAGAGCGCATCCGG
CTCCCCGATGACTGCACCATTGGCTACATTGTAGAGGCTCTGCTGGGTGTACCCCTC
ATCCGGAGCGGCCTCTTCCACTCCCACCTAGAGAACCTGCAGCAGGTGCCCACCACC
GAGCTTCATGAGCAGGTGACCCTGAGCTATGGCATGTTTGAGAACAAGCGGAACGCA
GTGCACATCAAGGGACCATTCTCTGTGGAAGCTGACCCATCCAGGTTCCGCTCTGTC
CATTGCCACCTGTACCCAGACACACCCTGGTGTCCTCGCTCCGCCATCTTCTAGCAG
TCGTGGTTGA

TABLE 1B

MLQRCGRRLLLALVGALLACLLVLTADPPPTPMPAERGRRALRTLAGSSGGAPASGS
RAAVDPGVLTREVHSLSEYFSLLTRARRDADPPPGVASRQGDGHPRPPAEVLSPRDV
FIAVKTTRKFHRARLDLLFETWISRHKEMTFIFTDGEDEALAKLTGNVVLTNCSSAH
SRQALSCKMAVEYDRFIESGKKWFCHVDDDNYVNLRALLRLLASYPHTQDVYIGKPS
LDRPIQATERISEHKVRPVHFWFATGGAGFCISRGLALKMGPWASGGHFMSTAERIR
LPDDCTIGYIVEALLGVPLIRSGLFHSHLENLQQVPTTELHEQVTLSYGMFENKRNA
VHIKGPFSVEADPSRFRSVHCHLYPDTPWCPRSAIF

TABLE 2A

ATGCACTGCCGACTTTTTCGGGGCATGGCGGGAGCCCTCTTTACCCTCCTGTGCGTG
GGGCTCCTGTCTCTACGATACCACTCAAGTTTGTCCCAGAGGATGATACAGGGCGCG
CTCAGGCTGAACCAACGGAACCCAGGACCCCTGGAGCTGCAGCTAGGCGACATCTTC
ATCGCAGTCAAGACTACCTGGGCCTTCCATCGCTCCCGCCTGGACCTGCTACTAGAC
ACGTGGGTCTCCAGGATCAGGCAACAGACATTCATCTTCACTGACAGCCCAGATGAA
CGCCTCCAGGAGAGACTAGGCCCGCACCTCGTGGTCACCAACTGTTCTGCAGAGCAC
AGTCATCCTGCTCTGTCCTGCAAGATGGCTGCAGAGTTCGATGCCTTCTTGGTCAGT
GGCCTCAGGTGGTTCTGCCACGTGGATGATGACAACTATGTGAACCCCAAGGCTCTG
CTGCAGCTGTTGAAAACATTCCCGCAGGACCGTGATGTCTATGTGGGCAAGCCCAGC
CTGAACCGGCCCATCCACGCCTCTGAGCTGCAGTCAAAAAACCGCACGAAGCTGGTG
CGGTTCTGGTTTGCCACAGGGGGTGCTGGTTTCTGCATCAACCGCCAACTGGCTTTG
AAGATGGTGCCATGGGCCAGCGGCTCCCACTTTGTGGACACTTCTGCTCTCATCCGG
CTCCCCGATGACTGCACTGTGGGCTACATCATCGAGTGCAAGCTGGGGGGTCGCCTG
CAGCCCAGCCCCCTCTTCCACTCACACCTGGAAACCCTGCAGCTGCTGGGGGCCGCC
CAGCTTCCGGAGCAGGTCACCCTCAGCTACGGTGTCTTTGAGGGGAAACTGAATGTC
ATCAAGCTACCGGGCCCCTTCTCCCATGAAGAGGACCCCTCCAGATTCCGCTCCCTC
CATTGTCTCCTCTACCCAGACACACCCTGGTGTCCGCTGCTGGCAGCGCCCTGA

TABLE 2B

MHCRLFRGMAGALFTLLCVGLLSLRYHSSLSQRMIQGALRLNQRNPGPLELQLGDIF
IAVKTTWAFHRSRLDLLLDTWVSRIRQQTFIFTDSPDERLQERLGPHLVVTNCSAEH
SHPALSCKMAAEFDAFLVSGLRWFCHVDDDNYVNPKALLQLLKTFPQDRDVYVGKPS
LNRPIHASELQSKNRTKLVRFWFATGGAGFCINRQLALKMVPWASGSHFVDTSALIR
LPDDCTVGYIIECKLGGRLQPSPLFHSHLETLQLLGAAQLPEQVTLSYGVFEGKLNV
IKLPGPFSHEEDPSRFRSLHCLLYPDTPWCPLLAAP

TABLE 3A

ATGAGCCGTGCGCGGCGGGTGTTGTGCCGGGCCTGCCTCGCGCTGGCCGCGGTCCTG
GCTGTGTTGCTGCTACTGCCGCTGCCGCTACCGCTGCCGCTGCCTCGCGCGCCCGCA
CCGGACCCCGATCGGGTCCCGACCCGGAGCCTGACCCTCGAGGGAGACCGCCTGCAA
CCCGACGACGTCTTCATTGCAGTCAAGACCACTCGGAAGAACCACGGCCCGCGCCTG
CGGCTGCTGCTGCGTACCTGGATCTCACGAGCCCCACGGCAGACGTTCATTTTCACC
GATGGAGACGACCCTGAGCTCCAGATGCTGGCAGGCGGCCGCATGATCAACACCAAT
TGCTCTGCTGTGCGCACCCGCCAAGCACTGTGCTGCAAAATGTCGGTGGAATATGAT
AAGTTCCTAGAATCTGGACGAAAATGGTTCTGCCACGTGGATGATGACAACTACGTG
AACCCCAAAAGCCTGCTGCACCTGCTTTCCACCTTCTCTTCCAACCAGGACATCTAC
CTGGGGCGACCTAGCCTGGACCACCCCATCGAAGCCACAGAGAGGGTCCAAGGCGGT
GGCACCTCAAACACAGTGAAATTCTGGTTTGCTACTGGTGGGGCTGGGTTCTGCCTG
AGCAGGGGCCTTGCCCTCAAAATGAGCCCGTGGGCCAGCCTTGGCAGTTTCATGAGC
ACAGCAGAGCGGGTTCGGCTGCCTGATGACTGCACTGTGGGATACATCGTGGAAGGA
CTTCTGGGCGCCCGTCTGCTCCATAGCCCCCTGTTCCACTCGCACCTGGAAAACCTG
CAGAGGCTGCCGTCTGGTGCTATTTTGCAGCAGGTTACCTTGAGCTATGGGGGTCCT
GAGAACCCACATAATGTGGTGAATGTAGCTGGCAGTTTCAACATACAGCAGGACCCT
ACACGGTTTCAGTCTGTGCACTGCCTTCTCTACCCAGACACCCACTGGTGTCCTATG
AAGAACAGGGTTGAGGGAGCTTTCCAGTAA

TABLE 3B

MSRARRVLCRACLALAAVLAVLLLLPLPLPLPLPRAPAPDPDRVPTRSLTLEGDRLQ
PDDVFIAVKTTRKNHGPRLRLLLLRTWISRAPRQTFIFTDGDDPELQMLAGGRMINTN
CSAVRTRQALCCKMSVEYDKFLESGRKWFCHVDDDNYVNPKSLLHLLSTFSSNQDIY
LGRPSLDHPIEATERVQGGGTSNTVKFWFATGGAGFCLSRGLALKMSPWASLGSFMS
TAERVRLPDDCTVGYIVEGLLGARLLHSPLFHSHLENLQRLPSGAILQQVTLSYGGP
ENPHNVVNVAGSFNIQQDPTRFQSVHCLLYPDTHWCPMKNRVEGAFQ

TABLE 4

```
                    10         20         30         40         50
D-Fringe          1 MMSLTVLSPP QRFKRILQAM MLAVAVVYMT LLLYQSAYGY PGIQVPHSQV      50
X-Lunatic         1 MLK------- NWGKKLLLSI ---VGATLTC LLV------- --LVVDQQSR      50
m-Lunatic         1 MLQ------- RCGRRLLLAL ---VGALLAC LLV------- --LTADPPPT      50
m-Manic           1 M--------- HC--RLFRGM ---AGALFT- LLC------- --VGL-----      50
m-Radical         1 M--------- ---SRARRVL ---CR---AC LAL------- --AAV-LAVL      50
X-Radical         1 M--------- ---KITYVGL ---IK---VC FLV------- --FLL-LCAT      50

                   60         70         80         90        100
D-Fringe         51 DALASEAVTT HRDQLLQDYV QSSTPTQPGA GAPAASPTTV IIRKDIRSFN     100
X-Lunatic        51 HMLETQSDHE PGSAAAVHLR ADLDPANPGD G---GDPA-- NSAQDSGTFS     100
m-Lunatic        51 PM-----PAE RGRRALRTLA GSSGGAPASG SRAAVDPG-- VLTREVHSLS     100
m-Manic          51 ---------- ------LSLR ---------- ---------- ----------     100
m-Radical        51 LLLP------ ---- -LP-- ---------- ---------- -LP---LPLP     100
X-Radical        51 VLLN------ ------ISWR QRDSSQSLQH CNSTCSAK-- YLE---TKLK     100

                  110        120        130        140        150
D-Fringe        101 FSDIEVSERP TATLLTELAR RSRNGELLRD LSQR-AVTAT PQPPVTEL--     150
X-Lunatic       101 ---------- --AYFNKLTR VRRDVEQVAA PSK------D SAAPEEDITA     150
m-Lunatic       101 ---------- --EYFSLLTR ARRDADPPPG VASRQ-GDGH PRPPAEVLSP     150
m-Manic         101 ---------- ---YHSSLS- -----QRMIQ GALRL-NQRN PGPLELQLG-     150
m-Radical       101 ---------- --RAPAPDPD ------RVPT RSLTLEG--- -----DRLQP     150
X-Radical       101 ---------- --EAHLTGRH KKWETYRLDA KPTSATGQGH QHFAKEPLQI     150

                  160        170        180        190        200
D-Fringe        151 DDIFISVKTT KNYHDTRLAL IIKTWFQLAR DQTWFFTDTD DHYYQEKTKG     200
X-Lunatic       151 NDVFIAVKTT KKFHRSRMDL LMDTWISRNK EQTFIFTDGE DEELQ-KKTG     200
m-Lunatic       151 RDVFIAVKTT RKFHRARLDL LFETWISRHK EMTFIFTDGE DEALA-KLTG     200
m-Manic         151 -DIFIAVKTT WAFHRSRLDL LLDTWVSRIR QQTFIFTDSP DERLQERLGP     200
m-Radical       151 DDVFIAVKTT RKNHGPRLRL LLRTWISRAP RQTFIFTDGD DPELQMLAGG     200
X-Radical       151 KDLFIAVKTT KKYHGNRLNL LMQTWISRAK EQTFIFTDWE DQELRQKAGD     200

                  210        220        230        240        250
D-Fringe        201 HLINTKCSQG HFRKALCCKM SAELDVFLES GKKWFCHFDD DNYVNVPRLV     250
X-Lunatic       201 NVISTNCSAA HSRQALSCKM AVEYDKFIES DKKWFCHVDD DNYVNVRTLV     250
m-Lunatic       201 NVVLTNCSSA HSRQALSCKM AVEYDRFIES GKKWFCHVDD DNYVNLRALL     250
m-Manic         201 HLVVTNCSAE HSHPALSCKM AAEFDAFLVS GLRWFCHVDD DNYVNPKALL     250
m-Radical       201 RMINTNCSAV RTRQALCCKM SVEYDKFLES GRKWFCHVDD DNYVNPKSLL     250
X-Radical       201 QMVNTNCSAV HTRQALCCKM AVEYDKFVLS DKKWFCHLDD DNYLNLHALL     250
                        *          *                    *

                  260        270        280        290        300
D-Fringe        251 KLLDEYSPSV DWYLGKPSIS SPLEIHLDSK NTTTNKKITF WFATGGAGFC     300
X-Lunatic       251 KLLSRYSHTN DIYIGKPSLD RPIQATERI- SESNMRPVNF WFATGGAGFC     300
m-Lunatic       251 RLLASYPHTQ DVYIGKPSLD RPIQATERI- SEHKVRPVHF WFATGGAGFC     300
m-Manic         251 QLLKTFPQDR DVYVGKPSLN RPIHASELQ- SKNRTKLVRF WFATGGAGFC     300
m-Radical       251 HLLSTFSSNQ DIYLGRPSLD HPIEATERVQ GGGTSNTVKF WFATGGAGFC     300
X-Radical       251 DLLSTFSHST DVYVGRPSLD HPVETVDRMK GDGSGS-LKF WFATGGAGFC     300
                                                             *

                  310        320        330        340        350
D-Fringe        301 LSRALTLKML PIAGGGKFIS IGDKIRFPDD VTMGFIIEHL LKVPLTVVDN     350
X-Lunatic       301 ISRGLALKMS PWASGGHFMN TAEKIRLPDD CTIGYIIESV LGVKLIRSNL     350
m-Lunatic       301 ISRGLALKMG PWASGGHFMS TAERIRLPDD CTIGYIVEAL LGVPLIRSGL     350
m-Manic         301 INRQLALKMV PWASGSHFVD TSALIRLPDD CTVGYIIECK LGGRLQPSPL     350
m-Radical       301 LSRGLALKMS PWASLGSFMS TAERVRLPDD CTVGYIVEGL LGARLLHSPL     350
X-Radical       301 ISRGLALKMS PWASMGNFIS TAEKVRLPDD CTIGYIIEGM LDVKMQHSNL     350

                  360        370        380        390        400
D-Fringe        351 FHSHLEPMEF IRQDTFQDQV SFSYAHMKNQ WNVIKVDG-F DMKTDPKRFY     400
X-Lunatic       351 FHSHLENLHQ VPQSEIHNQV TLSYGMFENK RNAILMKGAF SVEEDPSRFR     400
m-Lunatic       351 FHSHLENLQQ VPTTELHEQV TLSYGMFENK RNAVHIKGPF SVEADPSRFR     400
m-Manic         351 FHSHLETLQL LGAAQLPEQV TLSYGVFEGK LNVIKLPGPF SHEEDPSRFR     400
m-Radical       351 FHSHLENLQR LPSGAILQQV TLSYGGPENP HNVVNVAGSF NIQQDPTRFQ     400
X-Radical       351 FHSHLEHLQR LPTESLLKQV TLSYGGPDNK WNVVRVNGAF SLAEDPTRFK     400

                  410        420        430        440        450
D-Fringe        401 SLHCQLFPYF SFCPPR---- ---------- .......... ..........     450
X-Lunatic       401 SVHCLLYPDT PWCPWK---- -AAY...... .......... ..........     450
m-Lunatic       401 SVHCHLYPDT PWCPRS---- -AIF...... .......... ..........     450
m-Manic         401 SLHCLLYPDT PWCPLL---- -AAP...... .......... ..........     450
m-Radical       401 SVHCLLYPDT HWCPMKNRVE GAFQ...... .......... ..........     450
X-Radical       401 SVHCLLYSDT DWCP--NHKH NPTT...... .......... ..........     450
                      *          *
```

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: HSC RESEARCH AND DEVELOPMENT LIMITED PARTNERSHIP
(B) STREET: 555 UNIVERSITY AVENUE, SUITE 5270
(C) CITY: TORONTO
(D) STATE: ONTARIO
(E) COUNTRY: CANADA
(F) POSTAL CODE (ZIP): M5G 1X8
(G) TELEPHONE: 416 813 5982
(H) TELEFAX: 416 8137163

(ii) TITLE OF INVENTION: FRINGE PROTEINS AND NOTCH SIGNALLING

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: CA PCT/CA97/00775

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1150 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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

ATGCTCCAGC GGTGCGGCCG GCGCCTGCTG CTGGCGCTGG TGGGCGCGCT GTTGGCTTGT 60

CTCCTGGTGC TCACGGCCGA CCCGCCACCG ACTCCGATGC CCGCTGAGCG CGGACGGCGC 120

GCGCTGCGTA GCCTGGCGGG CTCCTCTGGA GGAGCTCCGG CTTCAGGGTC CAGGGCGGCT 180

GTGGATCCCG GAGTCCTCAC CCGCGAGGTG CATAGCCTCT CCGAGTACTT CAGTCTACTC 240

ACCCGCGCGC GCAGAGACGC GGATCCACCG CCCGGGGTCG CTTCTCGCCA GGGCGACGGC 300

CATCCGCGTC CCCCCGCCGA AGTTCTGTCC CCTCGCGACG TCTTCATCGC CGTCAAGACC 360

ACCAGAAAGT TTCACCGCGC GCGGCTCGAT CTGCTGTTCG AGACCTGGAT CTCGCGCCAC 420

AAGGAGATGA CGTTCATCTT CACTGATGGG GAGGACGAAG CTCTGGCCAA GCTCACAGGC 480

AATGTGGTGC TCACCAACTG CTCCTCGGCC CACAGCCGCC AGGCTCTGTC CTGCAAGATG 540

GCTGTGGAGT ATGACCGATT CATTGAGTCT GGGAAGAAGT GGTTCTGCCA CGTGGATGAT 600

GACAACTACG TCAACCTCCG GGCGCTGCTG CGGCTCCTGG CCAGCTATCC CCACACCCAA 660

GACGTGTACA TCGGCAAGCC CAGCCTGGAC AGGCCCATCC AGGCCACAGA ACGGATCAGC 720

GAGCACAAAG TGAGACCTGT CCACTTTTGG TTTGCCACCG GAGGAGCTGG CTTCTGCATC 780

AGCCGAGGGC TGGCCCTAAA GATGGGCCCA TGGGCCAGTG GAGGACACTT CATGAGCACG 840

GCAGAGCGCA TCCGGCTCCC CGATGACTGC ACCATTGGCT ACATTGTAGA GGCTCTGCTG 900

GGTGTACCCC TCATCCGGAG CGGCCTCTTC CACTCCCACC TAGAGAACCT GCAGCAGGTG 960

CCCACCACCG AGCTTCATGA GCAGGTGACC CTGAGCTATG GCATGTTTGA GAACAAGCGG 1020

AACGCAGTGC ACATCAAGGG ACCATTCTCT GTGGAAGCTG ACCCATCCAG GTTCCGCTCT 1080

GTCCATTGCC ACCTGTACCC AGACACACCC TGGTGTCCTC GCTCCGCCAT CTTCTAGCAG 1140

TCGTGGTTGA 1150

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 378 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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

Met Leu Gln Arg Cys Gly Arg Arg Leu Leu Leu Ala Leu Val Gly Ala
1 5 10 15

Leu Leu Ala Cys Leu Leu Val Leu Thr Ala Asp Pro Pro Pro Thr Pro
20 25 30

Met Pro Ala Glu Arg Gly Arg Arg Ala Leu Arg Thr Leu Ala Gly Ser
35 40 45

Ser Gly Gly Ala Pro Ala Ser Gly Ser Arg Ala Ala Val Asp Pro Gly
50 55 60

Val Leu Thr Arg Glu Val His Ser Leu Ser Glu Tyr Phe Ser Leu Leu
65 70 75 80

Thr Arg Ala Arg Arg Asp Ala Asp Pro Pro Pro Gly Val Ala Ser Arg
85 90 95

Gln Gly Asp Gly His Pro Arg Pro Pro Ala Glu Val Leu Ser Pro Arg
100 105 110

Asp Val Phe Ile Ala Val Lys Thr Thr Arg Lys Phe His Arg Ala Arg
115 120 125

Leu Asp Leu Leu Phe Glu Thr Trp Ile Ser Arg His Lys Glu Met Thr
130 135 140

Phe Ile Phe Thr Asp Gly Glu Asp Glu Ala Leu Ala Lys Leu Thr Gly
145 150 155 160

Asn Val Val Leu Thr Asn Cys Ser Ser Ala His Ser Arg Gln Ala Leu
165 170 175

Ser Cys Lys Met Ala Val Glu Tyr Asp Arg Phe Ile Glu Ser Gly Lys
180 185 190

Lys Trp Phe Cys His Val Asp Asp Asp Asn Tyr Val Asn Leu Arg Ala
195 200 205

Leu Leu Arg Leu Leu Ala Ser Tyr Pro His Thr Gln Asp Val Tyr Ile
210 215 220

Gly Lys Pro Ser Leu Asp Arg Pro Ile Gln Ala Thr Glu Arg Ile Ser
225 230 235 240

Glu His Lys Val Arg Pro Val His Phe Trp Phe Ala Thr Gly Gly Ala
245 250 255

Gly Phe Cys Ile Ser Arg Gly Leu Ala Leu Lys Met Gly Pro Trp Ala
260 265 270

Ser Gly Gly His Phe Met Ser Thr Ala Glu Arg Ile Arg Leu Pro Asp
275 280 285

Asp Cys Thr Ile Gly Tyr Ile Val Glu Ala Leu Leu Gly Val Pro Leu
290 295 300

Ile Arg Ser Gly Leu Phe His Ser His Leu Glu Asn Leu Gln Gln Val
305 310 315 320

Pro Thr Thr Glu Leu His Glu Gln Val Thr Leu Ser Tyr Gly Met Phe
325 330 335

Glu Asn Lys Arg Asn Ala Val His Ile Lys Gly Pro Phe Ser Val Glu
340 345 350

Ala Asp Pro Ser Arg Phe Arg Ser Val His Cys His Leu Tyr Pro Asp
355 360 365

Thr Pro Trp Cys Pro Arg Ser Ala Ile Phe
370 375

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 966 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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

ATGCACTGCC GACTTTTTCG GGGCATGGCG GGAGCCCTCT TTACCCTCCT GTGCGTGGGG 60

CTCCTGTCTC TACGATACCA CTCAAGTTTG TCCCAGAGGA TGATACAGGG CGCGCTCAGG 120

CTGAACCAAC GGAACCCAGG ACCCCTGGAG CTGCAGCTAG GCGACATCTT CATCGCAGTC 180

AAGACTACCT GGGCCTTCCA TCGCTCCCGC CTGGACCTGC TACTAGACAC GTGGGTCTCC 240

AGGATCAGGC AACAGACATT CATCTTCACT GACAGCCCAG ATGAACGCCT CCAGGAGAGA 300

CTAGGCCCGC ACCTCGTGGT CACCAACTGT TCTGCAGAGC ACAGTCATCC TGCTCTGTCC 360

TGCAAGATGG CTGCAGAGTT CGATGCCTTC TTGGTCAGTG GCCTCAGGTG GTTCTGCCAC 420

GTGGATGATG ACAACTATGT GAACCCCAAG GCTCTGCTGC AGCTGTTGAA AACATTCCCG 480

CAGGACCGTG ATGTCTATGT GGGCAAGCCC AGCCTGAACC GGCCCATCCA CGCCTCTGAG 540

CTGCAGTCAA AAAACCGCAC GAAGCTGGTG CGGTTCTGGT TTGCCACAGG GGGTGCTGGT 600

TTCTGCATCA ACCGCCAACT GGCTTTGAAG ATGGTGCCAT GGGCCAGCGG CTCCCACTTT 660

GTGGACACTT CTGCTCTCAT CCGGCTCCCC GATGACTGCA CTGTGGGCTA CATCATCGAG 720

TGCAAGCTGG GGGGTCGCCT GCAGCCCAGC CCCCTCTTCC ACTCACACCT GGAAACCCTG 780

CAGCTGCTGG GGGCCGCCCA GCTTCCGGAG CAGGTCACCC TCAGCTACGG TGTCTTTGAG 840

GGGAAACTGA ATGTCATCAA GCTACCGGGC CCCTTCTCCC ATGAAGAGGA CCCCTCCAGA 900

TTCCGCTCCC TCCATTGTCT CCTCTACCCA GACACACCCT GGTGTCCGCT GCTGGCAGCG 960

CCCTGA 966

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 321 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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

Met His Cys Arg Leu Phe Arg Gly Met Ala Gly Ala Leu Phe Thr Leu
1 5 10 15

Leu Cys Val Gly Leu Leu Ser Leu Arg Tyr His Ser Ser Leu Ser Gln
20 25 30

Arg Met Ile Gln Gly Ala Leu Arg Leu Asn Gln Arg Asn Pro Gly Pro
35 40 45

Leu Glu Leu Gln Leu Gly Asp Ile Phe Ile Ala Val Lys Thr Thr Trp
50 55 60

Ala Phe His Arg Ser Arg Leu Asp Leu Leu Leu Asp Thr Trp Val Ser
65 70 75 80

Arg Ile Arg Gln Gln Thr Phe Ile Phe Thr Asp Ser Pro Asp Glu Arg
85 90 95

Leu Gln Glu Arg Leu Gly Pro His Leu Val Val Thr Asn Cys Ser Ala
100 105 110

Glu His Ser His Pro Ala Leu Ser Cys Lys Met Ala Ala Glu Phe Asp
115 120 125

Ala Phe Leu Val Ser Gly Leu Arg Trp Phe Cys His Val Asp Asp Asp
130 135 140

Asn Tyr Val Asn Pro Lys Ala Leu Leu Gln Leu Leu Lys Thr Phe Pro
145 150 155 160

Gln Asp Arg Asp Val Tyr Val Gly Lys Pro Ser Leu Asn Arg Pro Ile
165 170 175

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

Trp Phe Ala Thr Gly Gly Ala Gly Phe Cys Ile Asn Arg Gln Leu Ala
195 200 205

Leu Lys Met Val Pro Trp Ala Ser Gly Ser His Phe Val Asp Thr Ser
210 215 220

Ala Leu Ile Arg Leu Pro Asp Asp Cys Thr Val Gly Tyr Ile Ile Glu
225 230 235 240

Cys Lys Leu Gly Gly Arg Leu Gln Pro Ser Pro Leu Phe His Ser His
245 250 255

Leu Glu Thr Leu Gln Leu Leu Gly Ala Ala Gln Leu Pro Glu Gln Val
260 265 270

Thr Leu Ser Tyr Gly Val Phe Glu Gly Lys Leu Asn Val Ile Lys Leu
275 280 285

Pro Gly Pro Phe Ser His Glu Glu Asp Pro Ser Arg Phe Arg Ser Leu
290 295 300

His Cys Leu Leu Tyr Pro Asp Thr Pro Trp Cys Pro Leu Leu Ala Ala
305 310 315 320

Pro

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 999 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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

ATGAGCCGTG CGCGGCGGGT GTTGTGCCGG GCCTGCCTCG CGCTGGCCGC GGTCCTGGCT 60

GTGTTGCTGC TACTGCCGCT GCCGCTACCG CTGCCGCTGC CTCGCGCGCC CGCACCGGAC 120

CCCGATCGGG TCCCGACCCG GAGCCTGACC CTCGAGGGAG ACCGCCTGCA ACCCGACGAC 180

GTCTTCATTG CAGTCAAGAC CACTCGGAAG AACCACGGCC CGCGCCTGCG GCTGCTGCTG 240

CGTACCTGGA TCTCACGAGC CCCACGGCAG ACGTTCATTT TCACCGATGG AGACGACCCT 300

GAGCTCCAGA TGCTGGCAGG CGGCCGCATG ATCAACACCA ATTGCTCTGC TGTGCGCACC 360

CGCCAAGCAC TGTGCTGCAA AATGTCGGTG GAATATGATA AGTTCCTAGA ATCTGGACGA 420

AAATGGTTCT GCCACGTGGA TGATGACAAC TACGTGAACC CCAAAAGCCT GCTGCACCTG 480

CTTTCCACCT TCTCTTCCAA CCAGGACATC TACCTGGGGC GACCTAGCCT GGACCACCCC 540

ATCGAAGCCA CAGAGAGGGT CCAAGGCGGT GGCACCTCAA ACACAGTGAA ATTCTGGTTT 600

GCTACTGGTG GGGCTGGGTT CTGCCTGAGC AGGGGCCTTG CCCTCAAAAT GAGCCCGTGG 660

GCCAGCCTTG GCAGTTTCAT GAGCACAGCA GAGCGGGTTC GGCTGCCTGA TGACTGCACT 720

GTGGGATACA TCGTGGAAGG ACTTCTGGGC GCCCGTCTGC TCCATAGCCC CCTGTTCCAC 780

TCGCACCTGG AAAACCTGCA GAGGCTGCCG TCTGGTGCTA TTTTGCAGCA GGTTACCTTG 840

AGCTATGGGG GTCCTGAGAA CCCACATAAT GTGGTGAATG TAGCTGGCAG TTTCAACATA 900

CAGCAGGACC CTACACGGTT TCAGTCTGTG CACTGCCTTC TCTACCCAGA CACCCACTGG 960

TGTCCTATGA AGAACAGGGT TGAGGGAGCT TTCCAGTAA 999

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 332 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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

Met Ser Arg Ala Arg Arg Val Leu Cys Arg Ala Cys Leu Ala Leu Ala
1 5 10 15

Ala Val Leu Ala Val Leu Leu Leu Leu Pro Leu Pro Leu Pro Leu Pro
20 25 30

Leu Pro Arg Ala Pro Ala Pro Asp Pro Asp Arg Val Pro Thr Arg Ser
35 40 45

Leu Thr Leu Glu Gly Asp Arg Leu Gln Pro Asp Asp Val Phe Ile Ala
50 55 60

Val Lys Thr Thr Arg Lys Asn His Gly Pro Arg Leu Arg Leu Leu Leu
65 70 75 80

Arg Thr Trp Ile Ser Arg Ala Pro Arg Gln Thr Phe Ile Phe Thr Asp
85 90 95

Gly Asp Asp Pro Glu Leu Gln Met Leu Ala Gly Gly Arg Met Ile Asn
100 105 110

Thr Asn Cys Ser Ala Val Arg Thr Arg Gln Ala Leu Cys Cys Lys Met

115 120 125

Ser Val Glu Tyr Asp Lys Phe Leu Glu Ser Gly Arg Lys Trp Phe Cys
130 135 140

His Val Asp Asp Asp Asn Tyr Val Asn Pro Lys Ser Leu Leu His Leu
145 150 155 160

Leu Ser Thr Phe Ser Ser Asn Gln Asp Ile Tyr Leu Gly Arg Pro Ser
165 170 175

Leu Asp His Pro Ile Glu Ala Thr Glu Arg Val Gln Gly Gly Gly Thr
180 185 190

Ser Asn Thr Val Lys Phe Trp Phe Ala Thr Gly Gly Ala Gly Phe Cys
195 200 205

Leu Ser Arg Gly Leu Ala Leu Lys Met Ser Pro Trp Ala Ser Leu Gly
210 215 220

Ser Phe Met Ser Thr Ala Glu Arg Val Arg Leu Pro Asp Asp Cys Thr
225 230 235 240

Val Gly Tyr Ile Val Glu Gly Leu Leu Gly Ala Arg Leu Leu His Ser
245 250 255

Pro Leu Phe His Ser His Leu Glu Asn Leu Gln Arg Leu Pro Ser Gly
260 265 270

Ala Ile Leu Gln Gln Val Thr Leu Ser Tyr Gly Gly Pro Glu Asn Pro
275 280 285

His Asn Val Val Asn Val Ala Gly Ser Phe Asn Ile Gln Gln Asp Pro
290 295 300

Thr Arg Phe Gln Ser Val His Cys Leu Leu Tyr Pro Asp Thr His Trp
305 310 315 320

Cys Pro Met Lys Asn Arg Val Glu Gly Ala Phe Gln
325 330

CLAIMS

We claim:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a mammalian Fringe protein.

2. The nucleic acid of claim 1 comprising a nucleotide sequence selected from the group consisting of

(a) a sequence encoding a protein comprising the amino acid sequence of Table 1B (Sequence ID NO:2);

(b) a sequence encoding a protein comprising the amino acid sequence of Table 2B (Sequence ID NO:4);

(c) a sequence encoding a protein comprising the amino acid sequence of Table 3B (Sequence ID NO:6); and

(d) a sequence encoding a mammalian Fringe protein and capable of hybridising to a sequence complementary to any sequence of (a) to (c) under stringent hybridisation conditions.

3. The nucleic acid of claim 2 comprising a nucleotide sequence selected from the group consisting of

(a) the nucleotide sequence of Table 1A (Sequence ID No:1);

(b) the nucleotide sequence of Table 2A (Sequence ID No:3);

(c) the nucleotide sequence of Table 3A (Sequence ID No:5); and

(d) a nucleotide sequence complementary to a sequence of (a) to (c).

4. The nucleic acid of claim 1 encoding a murine Fringe protein.

5. A substantially pure mammalian Fringe protein.

6. The protein of claim 5 wherein the protein is encoded by a nucleic acid of any of claims 1 to 4.

7. The protein of claim 6 comprising an amino acid sequence selected from the group consisting of

(a) the amino acid sequence of Sequence ID NO:2;

(b) the amino acid sequence of Sequence ID NO:4; and

(c) the amino acid sequence of Sequence ID NO:6;

8. A substantially pure polypeptide comprising an amino acid sequence selected from the group consisting of

(a) at least 5 consecutive amino acid residues of a protein of any of claims 5 to 7;

(b) at least 10 consecutive amino acid residues of a protein of any of claims 5 to 7; and

(c) at least 15 consecutive amino acid residues of a protein of any of claims 5 to 7.

9. An immunogen comprising a portion of a protein of any of claims 5 to 7.

10. A recombinant vector comprising a nucleic acid of any of claims 1 to 4.

11. A host cell comprising a vector of claim 10.

12. A method of producing a mammalian Fringe protein comprising culturing a host cell of claim 11 under conditions suitable for expression of said protein and isolating said protein from said culture.

13. A purified antibody which selectively binds to an antigenic determinant of a mammalian Fringe protein.

14. The antibody of claim 13 wherein the protein is a protein of any of claims 5 to 7.

15. A non-human transgenic animal wherein a genome of said animal, or of an ancestor thereof, has been modified by introduction of a modification selected from the group consisting of

(a) insertion of a nucleotide sequence encoding a heterospecific Fringe protein;

(b) insertion of a nucleotide sequence encoding a dominant negative mutant of a Fringe protein; and

(c) inactivation of an endogenous Fringe gene.

16. A pharmaceutical composition comprising an active ingredient selected from the group consisting of

(a) a substantially pure mammalian Fringe protein;

(b) an isolated nucleic acid comprising a nucleotide sequence encoding a mammalian Fringe protein;

(c) an expression vector operably encoding a Fringe antisense sequence;

(d) an expression vector operably encoding a mammalian Fringe protein; and

(e) a substantially pure antibody, wherein the antibody selectively binds to an antigenic determinant of a mammalian Fringe protein

and a pharmaceutically acceptable carrier.

17. A method of preventing or treating a disorder in a mammal characterised by an abnormality in a signal transduction pathway which involves an interaction between a Notch receptor and a Notch ligand comprising modulating the Notch receptor/Notch ligand interaction by administration to the mammal of an effective amount of a mammalian Fringe protein or of an effective fragment or analogue thereof.

18. The method of claim 17 wherein the mammalian Fringe protein is Lunatic Fringe, Manic Fringe or Radical Fringe.

19. The method of claim 17 wherein the Notch ligand is Delta protein.

20. The method of claim 17 wherein the Notch ligand is Serrate protein.

21. The method of claim 17 wherein the mammalian Fringe protein is encoded by a nucleic acid of any of claims 1 to 4.

22. The method of claim 17 wherein the mammalian Fringe protein is a protein of any of claims 5 to 7.

23. The method of claim 17 wherein the disorder is selected from the group consisting of cancer,

cardiovascular disease, restenosis, and atherosclerosis.

24. A method for promoting differentiation of a mammalian cell by suppressing expression of Lunatic Fringe protein in the cell and/or promoting expression of Radical Fringe protein and/or Manic fringe protein in the cell.

25. A method for suppressing differentiation of a mammalian cell by suppressing expression of Radical Fringe protein and/or Manic Fringe protein in the cell and/or promoting expression of Lunatic Fringe protein in the cell.

26. A method for identifying compounds which modulate the expression of a mammalian Fringe gene comprising

contacting a cell with a candidate compound wherein said cell includes a regulatory region of a mammalian Fringe gene operably joined to a coding region; and

detecting a change in expression of said coding region.

27. A method for identifying compounds which can selectively bind to a mammalian Fringe protein comprising

providing a preparation including at least one mammalian Fringe protein;

contacting the preparation with a candidate compound; and

determining binding of the Fringe protein to the compound.

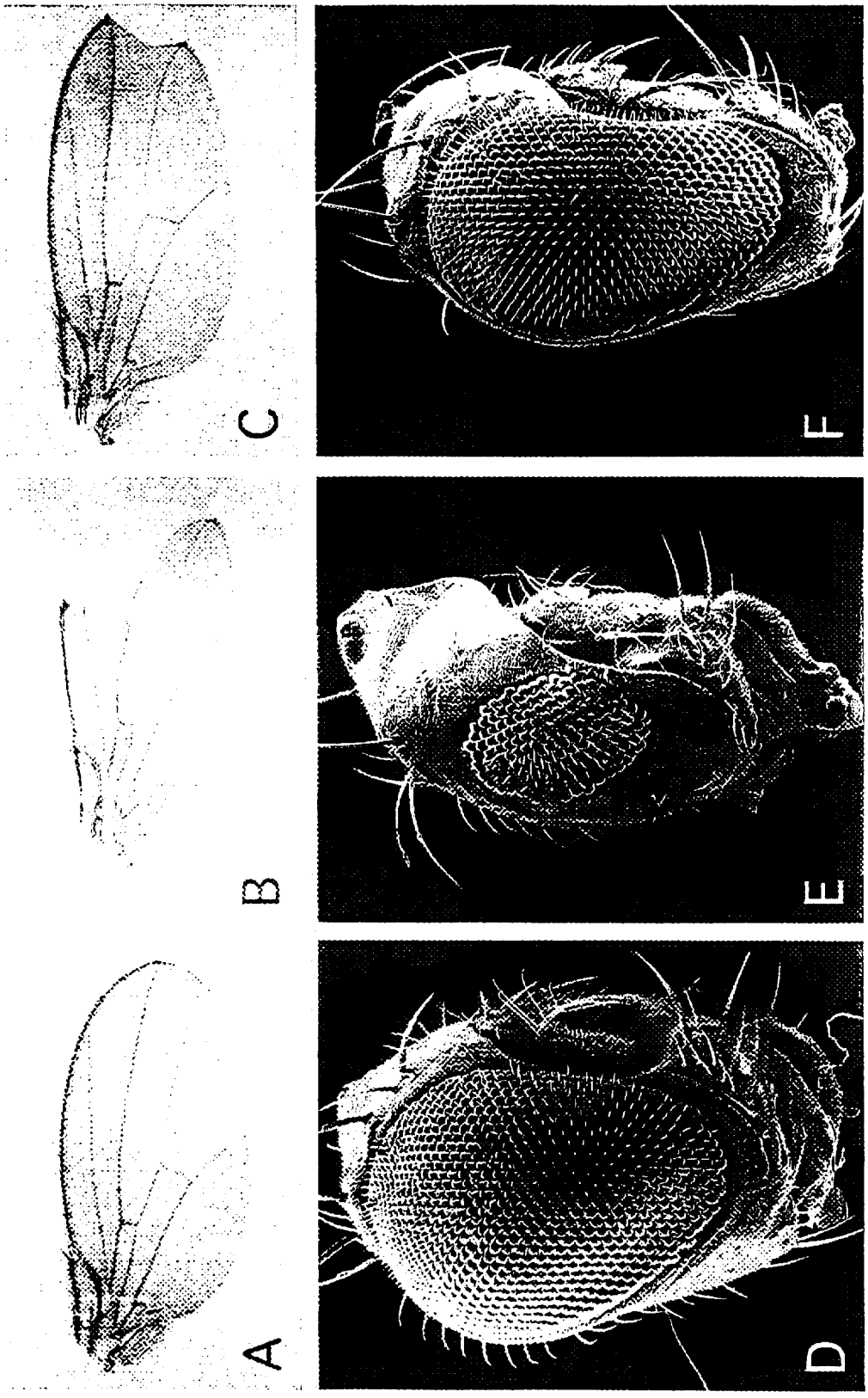


Figure 1A

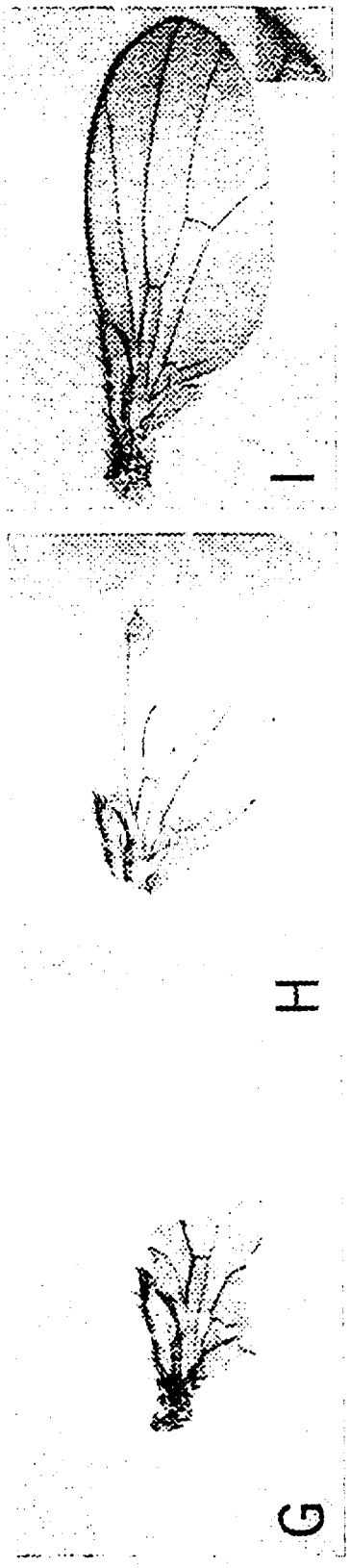


Figure 1B

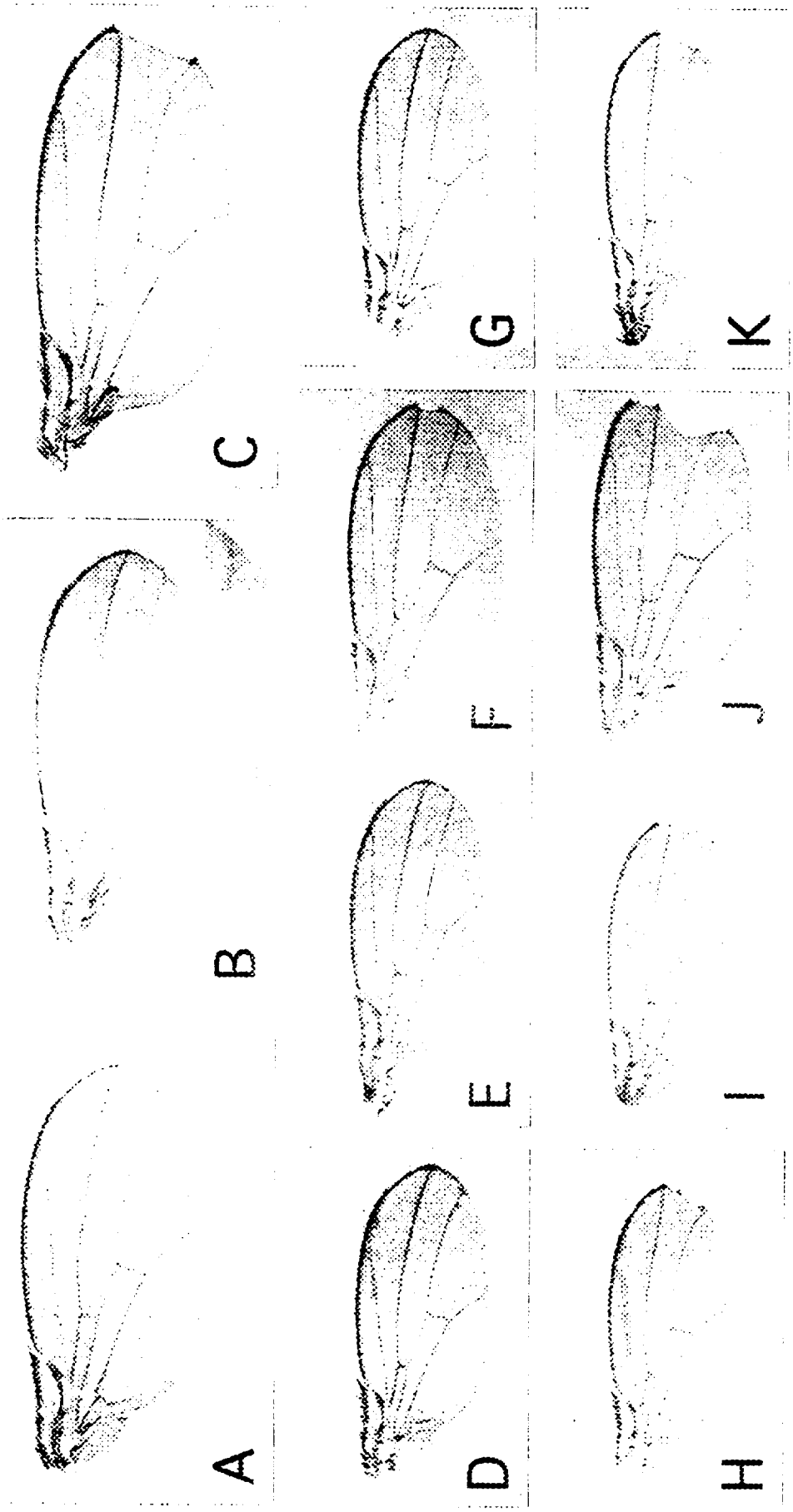


Figure 2

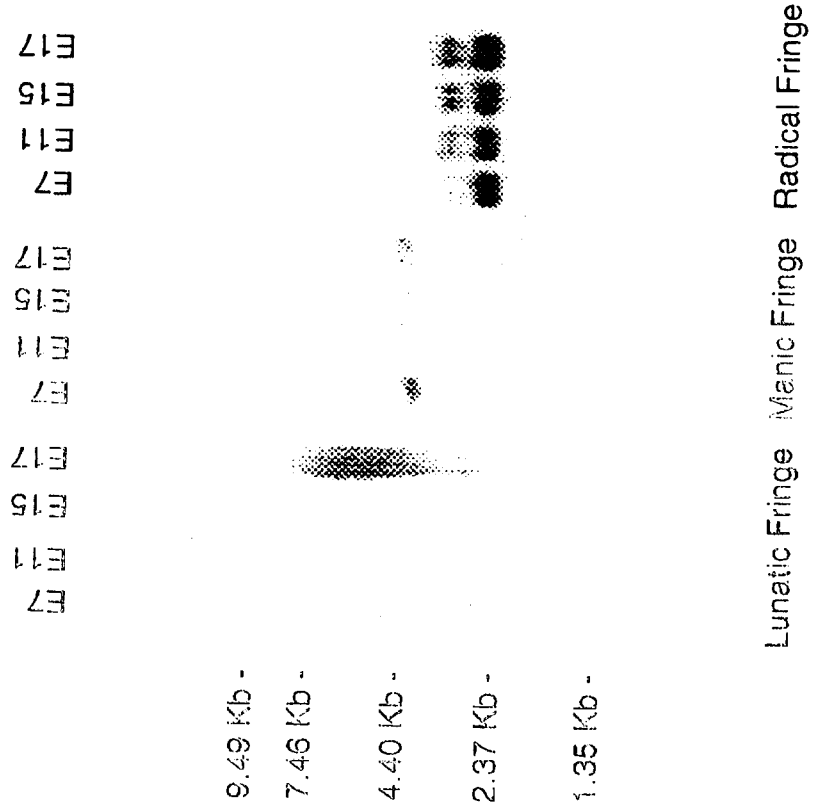

E17
E15
E11
E7
E17
E15
E11
E7
E17
E15
E11
E7
9.49 Kb -
7.46 Kb -
4.40 Kb -
2.37 Kb -
1.35 Kb -
Lunatic Fringe
Manic Fringe
Radical Fringe

Figure 3

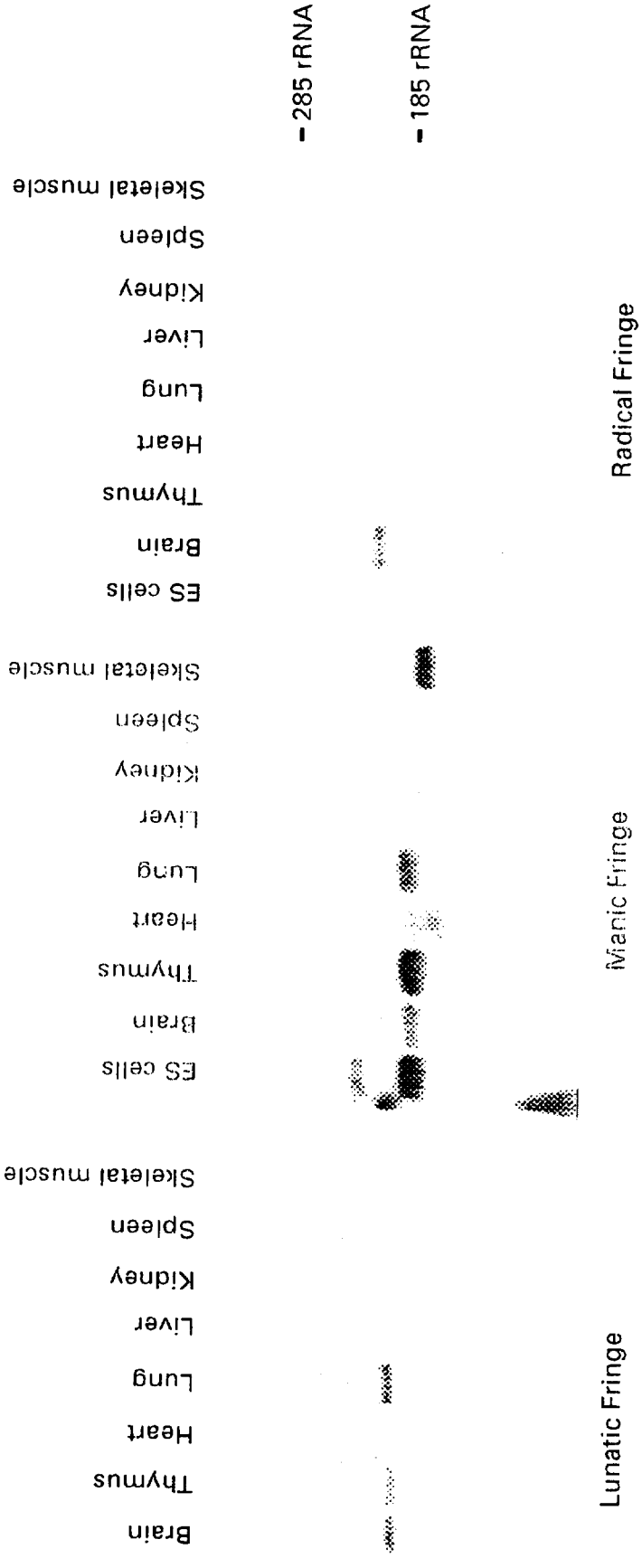
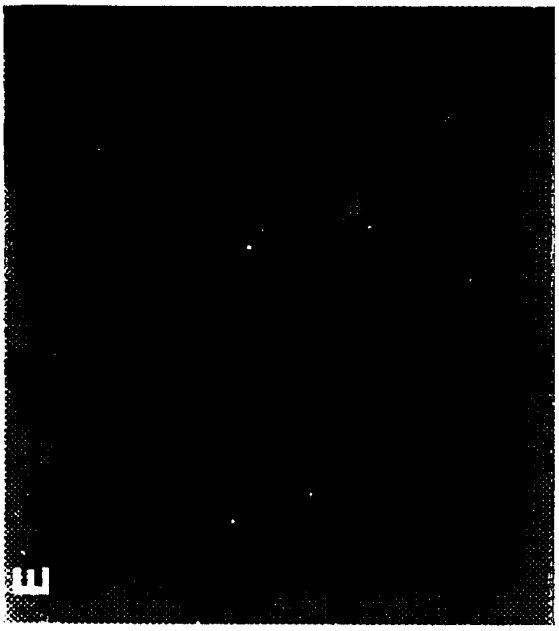

- 285 rRNA
- 185 rRNA
Skeletal muscle
Spleen
Kidney
Liver
Lung
Heart
Thymus
Brain
ES cells
Radical Fringe
Skeletal muscle
Spleen
Kidney
Liver
Lung
Heart
Thymus
Brain
ES cells
Manic Fringe
Skeletal muscle
Spleen
Kidney
Liver
Lung
Heart
Thymus
Brain
ES cells
Lunatic Fringe

Figure 4







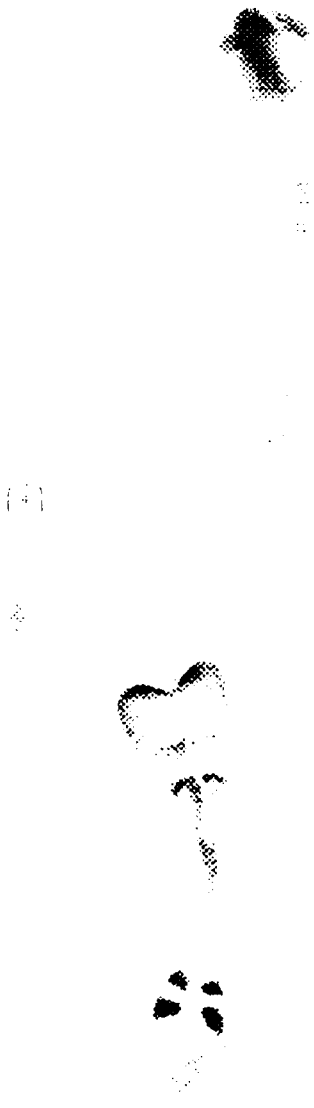




Figure 6A

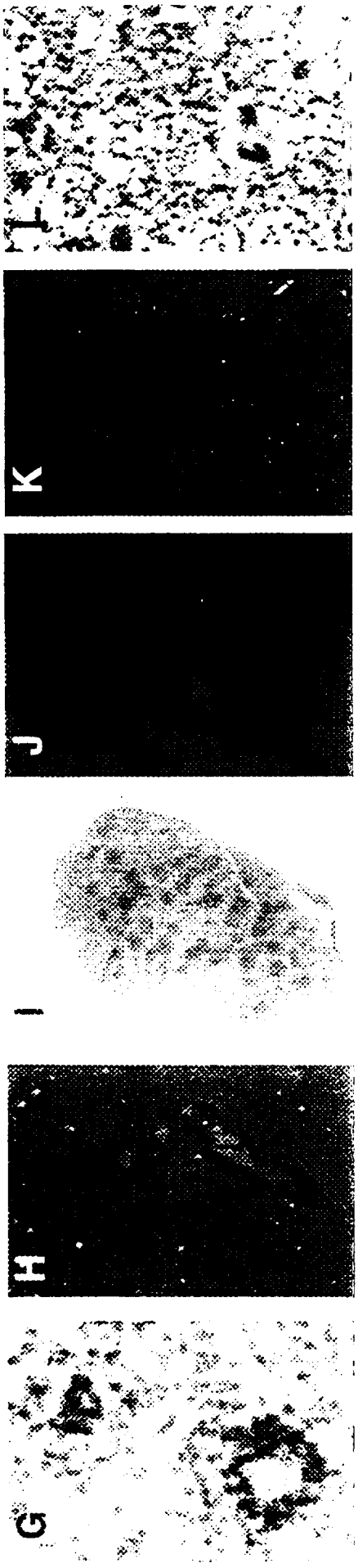


Figure 5B

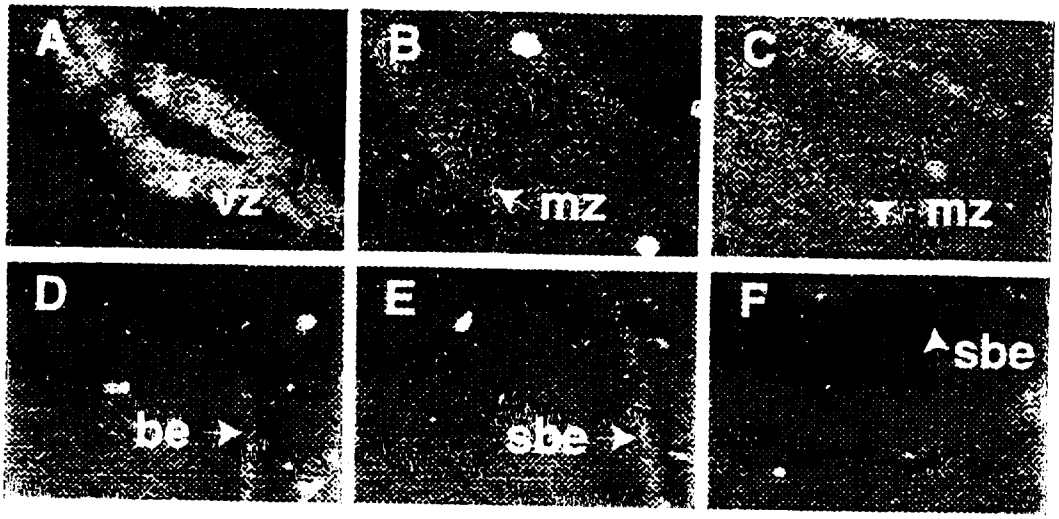


FIGURE 6

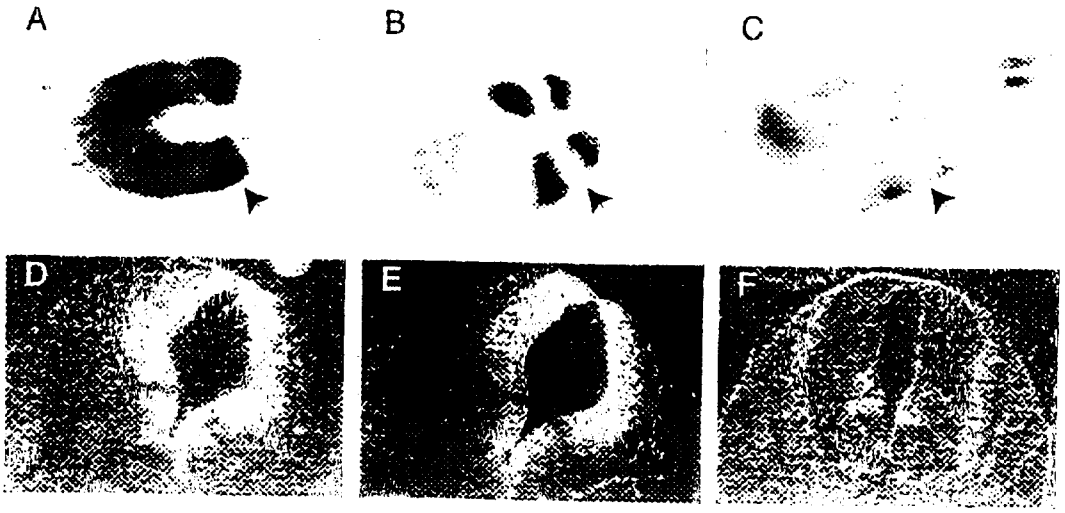


FIGURE 7

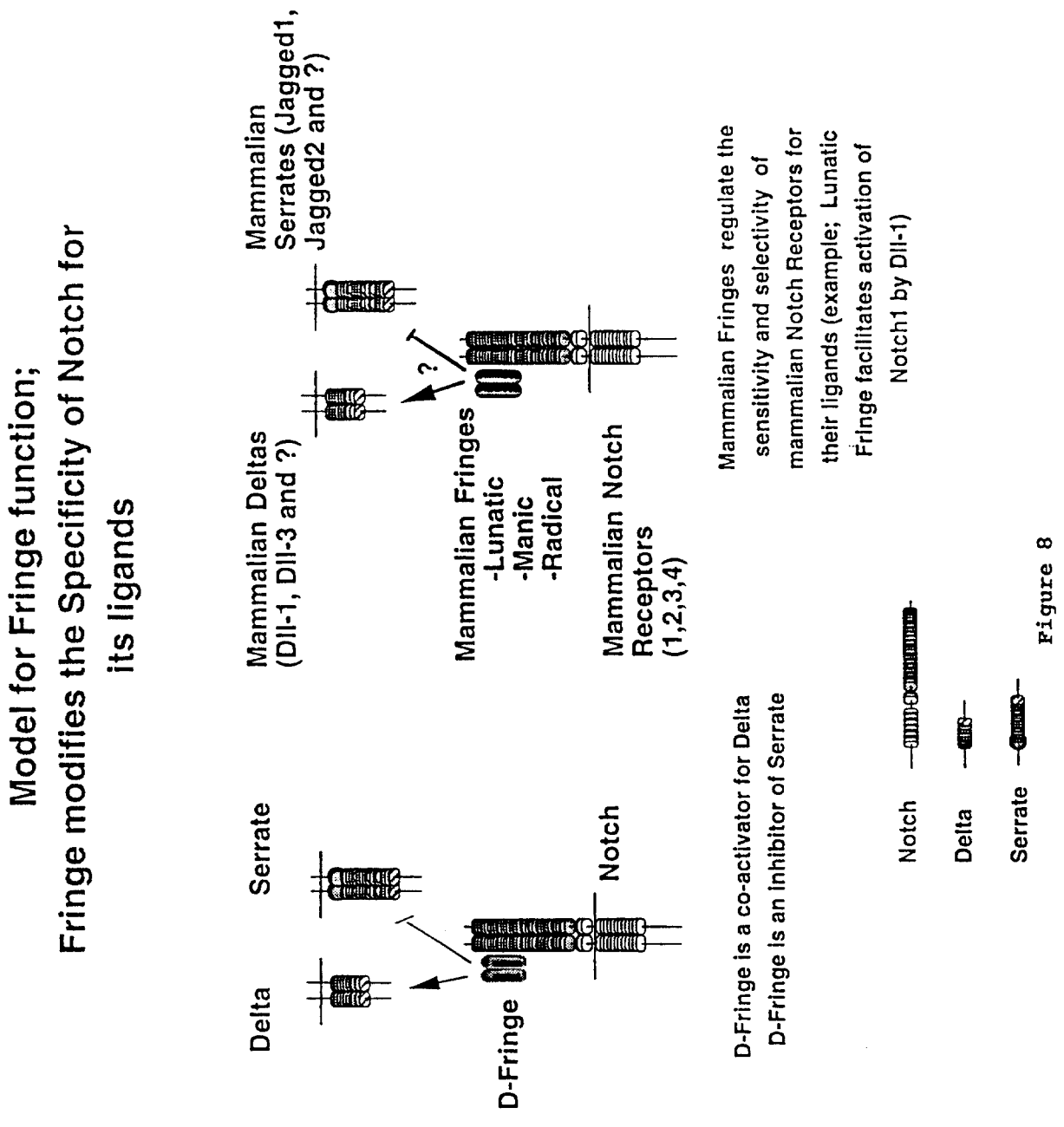

Model for Fringe function;
Fringe modifies the Specificity of Notch for its ligands
Delta
Serrate
D-Fringe
Notch
Mammalian Deltas (Dll-1, Dll-3 and ?)
Mammalian Serrates (Jagged1, Jagged2 and ?)
?
Mammalian Fringes
-Lunatic
-Manic
-Radical
Mammalian Notch Receptors (1,2,3,4)
D-Fringe is a co-activator for Delta
D-Fringe is an inhibitor of Serrate
Mammalian Fringes regulate the sensitivity and selectivity of mammalian Notch Receptors for their ligands (example; Lunatic Fringe facilitates activation of Notch1 by Dll-1)
Notch
Delta
Serrate

Figure 8

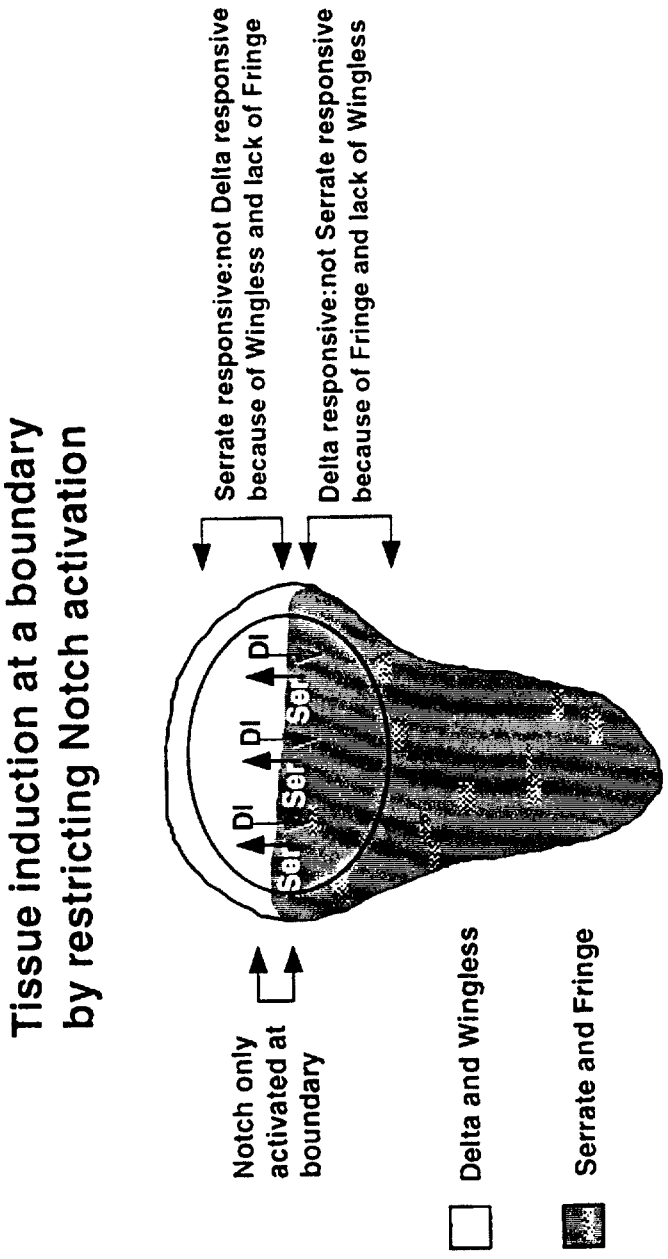
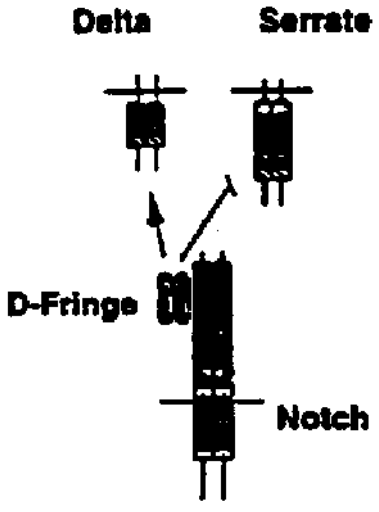
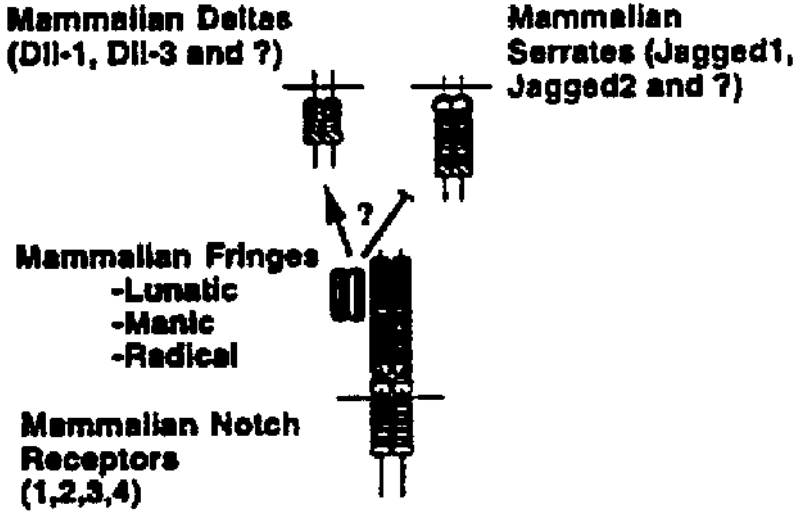

Tissue induction at a boundary
by restricting Notch activation
Serrate responsive:not Delta responsive
because of Wingless and lack of Fringe
Delta responsive:not Serrate responsive
because of Fringe and lack of Wingless
Dl
Ser
Notch only
activated at
boundary
Delta and Wingless
Serrate and Fringe

Figure 9

Model for Fringe function; Fringe modifies the Specificity of Notch for its ligands



D-Fringe is a co-activator for Delta
D-Fringe is an inhibitor of Serrate



Mammalian Fringes regulate the sensitivity and selectivity of mammalian Notch Receptors for their ligands (example: Lunatic Fringe facilitates activation of Notch1 by Dll-1)

