A Large Family of Putative Transmembrane Receptors Homologous to the Product of the Drosophila Tissue Polarity Gene frizzled*

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Random Sequencing of Retina cDNA Clones—From an adult human retina cDNA library in a gt10 (19), DNA inserts were isolated en mass by EcoRI cleavage and preparative agarose gel electrophoresis. To sample different regions of each cDNA and to generate smaller DNA segments for reproducible and high efficiency template preparation by

EXPERIMENTAL PROCEDURES

In Drosophila melanogaster, the frizzled gene plays an essential role in the development of tissue polarity as assessed by the orientation of cuticular structures. Through a combination of random cDNA sequencing, degenerate polymerase chain reaction amplification, and long stringency hybridization we have identified six novel frizzled homologues from mammals, at least 11 from zebrafish, several from chicken and sea urchin, and one from Caenorhabditis elegans. The complete deduced amino acid sequences of the mamalian and nematode homologues share with the Drosophila frizzled protein a conserved amino-terminal cysteine-rich domain and seven putative transmembrane segments. Each of the mamalian homologues is expressed in a distinctive set of tissues in the adult, and at least three are expressed during embryogenesis. As hypothesized for the Drosophila frizzled protein, the frizzled homologues are likely to act as transmembrane receptors for as yet unidentified ligands. These observations predict the existence of a family of signal transduction pathways that are homologous to the pathway that determines tissue polarity in Drosophila.

Much of animal development is orchestrated through the transmission of intercellular signals. In Drosophila melanogaster, genetic approaches have identified both receptors and ligands that mediate a number of these intercellular signaling processes. Examples include the receptors Sevenless (1), Notch (2), torso (3), and Toll (4), and the ligands Hedgehog (5), and wingless (6, 7). For several of these Drosophila proteins, vertebrate homologues have been identified, and in each case the homologue(s) appear to play important roles in development (8–11). These studies demonstrate a remarkable conservation of biochemical mechanisms between vertebrate and invertebrate cell-cell communication pathways despite differences in the morphologies of the corresponding organisms.

In Drosophila, as in other insects, the cuticle contains a variety of specialized structures, such as bristle sense organs and hairs. At each surface location these extended structures exhibit a predetermined orientation with respect to the body axes. For example, on the wings and legs they point away from the body, and on the thorax and abdomen they point posteriorly. Mutations that alter the polarity of these structures define a set of genes that are referred to as tissue polarity genes (12). Mutations in frizzled, the most extensively studied tissue polarity gene, disrupt to various extents the polarity of hairs on the wing, leg, thorax, and abdomen (13). Most alleles also disrupt the orderly arrangement of eye bristles, giving a rough eye phenotype. Genetic mosaic experiments in which homogenous frizzled/ frizzled patches are generated in a heterozygous background show that for some alleles the loss of tissue polarity is confined to the mutant patch, whereas for other alleles the loss of tissue polarity also spreads to adjacent cells distal to the mutant patch (14). These experiments have led Adler and colleagues (14) to propose that the frizzled gene product may be involved in both sending and receiving polarity information.

Strong support for a model in which frizzled acts directly in tissue polarity signaling comes from the sequence of the frizzled gene (15, 16). This sequence reveals a deduced protein product of 587 amino acids that begins with a putative signal peptide and contains seven putative transmembrane segments. Immunostaining of cells expressing frizzled cDNA shows that the protein accumulates in the plasma membrane and that the amino and carboxyl termini face the extracellular and intracellular spaces, respectively (17). Although the frizzled protein shares no primary sequence homology with any known receptor, its role in development and its plasma membrane location strongly suggest that it acts as a receptor, a ligand, or both in the transmission of tissue polarity information.

The goal of the present work is to determine the extent to which frizzled-like proteins exist outside of Drosophila. Previous work by Chan et al. (18) identified two closely related rat genes, the protein products of which share approximately 40% amino acid identity with the Drosophila frizzled protein. These genes were identified serendipitously, and their biological functions are currently unknown. In the present work, we show that at least one frizzled-like gene exists in the nematode, Caenorhabditis elegans, and that a large number of frizzled-like genes exist in vertebrates. These observations predict the existence of a family of signal transduction pathways homologous to the pathway that determines tissue polarity in Drosophila.

EXPERIMENTAL PROCEDURES

Random Sequencing of Retina cDNA Clones—From an adult human retina cDNA library in a gt10 (19), DNA inserts were isolated en mass by EcoRI cleavage and preparative agarose gel electrophoresis. To sample different regions of each cDNA and to generate smaller DNA segments for reproducible and high efficiency template preparation by
Table I

| Name | Strand | Amino acid sequence | Position of NH2-terminal amino acid |
|------|--------|---------------------|-----------------------------------|
| YW157 | Sense | YPERPI | 277 |
| YW158 | Antisense | WFLAA | 362 |
| YW164 | Sense | WWWIL | 354 |
| YW278 | Sense | (V.I.L).C(F.V.Y.G) | 411 |
| YW279 | Sense | M(V.I).F.R(K) | 496 |
| YW280 | Antisense | CY(Y.L).VYE | 489 |
| YW282 | Sense | Y(T.F).L(V.Y.D) | 267 |
| YW284 | Sense | M(I.L).V.K(Y.L).F | 535 |
| YW285 | Sense | M(V.I).F.R(K) | 469 |

PCR, the cDNA inserts were processed in either of two ways. In the first method, the cDNA inserts were used as a template for DNA synthesis with the Klenow fragment of Escherichia coli DNA polymerase I. The primer 5′-CCCCCCCCCGACGAGATATTAGAATTCTACTCGNNNNNN (where N indicates a mixture of all four bases) was used to prime PCR amplification (and a second round of priming with the same primer), those DNA segments carrying the primer sequence at both ends were PCR amplified with the primer 5′-CCCCCCCCCGACGAGATATTAGAATTCTACTCGNNNNNN as a probe. Following hybridization and a second round of priming with the same primer, those DNA segments carrying the primer sequence at both ends were PCR amplified with the primer 5′-CCCCCCCCCGACGAGATATTAGAATTCTACTCGNNNNNN as a probe. PCR products were sequenced as described above for random cDNA sequencing. RNAase Protection—Total RNA was prepared from adult mouse brain, eye, heart, kidney, liver, lung, spleen, and testes by homogenization in guanidinium thiocyanate and phenol extraction, followed by centrifugation through 5 M cesium chloride (23). Ten micrograms of total RNA from each tissue or of yeast RNA was used for the RNAase protection assay. Riboprobes were synthesized using either T7 or T3 RNA polymerase on linearized templates in pBluescript. Each mouse frizzled probe contained 150–250 bases from the antisense strand linker to 25–50 bases of vector sequence. Reagents were obtained from Ambion (Austin, TX), and the hybridization and digestion conditions were as recommended by Ambion.

The following table provides information on the probes and RFLPs for nearly all the loci linked to the frizzled genes has been reported. These include Glis, Ctlai4, and F1n1 on chromosome 1 (26, 27), Gnl1 and En2 on chromosome 5 (28); Tyr on chromosome 7 (29); Csf1 and Wnt3 on chromosome 11 (30); Ctlai1, Gya3, and Nf1 on chromosome 14 (31, 32); and Hspg3 and Myc on chromosome 15 (33). One locus, olfactory marker protein (Omp), has not been reported previously for the Frederick IB. The probe was kindly provided by Dr. Randall Reed and it detected a 2.2-kb BamHI fragment in C57BL/6J DNA and 3.3-kb BamHI fragment in M. spretus DNA. Restriction enzyme digestion distances were calculated as described previously (34). The mouse program SPRETUS MADNESS. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

In situ Hybridization—Freshly dissected adult brains, whole embryos, or heads were rapidly frozen in plastic molds placed on a dry ice/acetone slurry and processed for sectioning as described previously (35). 32P-labeled antisense riboprobes were prepared from linearized pBluescript plasmid subclones using either T3 or T7 RNA polymerase. In situ hybridization was performed as described by Saffron et al. (36), and hybridized sections were exposed to x-ray film and digitized.

RESULTS

Identification of New Members of the Frizzled Family—In an ongoing effort to identify novel genes expressed in the mammalian retina, we have determined partial sequences from 5,000 human retina cDNA clones. Conceptual translation of one of these sequences showed statistically significant similarity to the protein product of the Drosophila tissue polarity gene frizzled (15), and to two frizzled-related sequences of unknown function previously identified in the rat, Rfz1 and Rfz2 (18).

This partial cDNA sequence, hereafter referred to as Hfz3, showed approximately 40% amino acid identity with each of the three previously reported frizzled sequences. (Throughout
In this paper the species source of each frizzled sequence will be referred to by the first letter(s) of its abbreviated name: H, human; M, mouse; D, Drosophila; R, rat; Z, zebrafish; C, C. elegans; Ch, chicken; S, sea urchin.

To search for additional frizzled family members, we used PCR with different pairs of degenerate primers (Table I) to amplify genomic DNA and cDNA from both vertebrates and invertebrates. Alignment of the Drosophila, rat, and partial human sequences revealed two regions of amino acid identity that were used to design a first set of degenerate PCR primers, YW-157 and YW-158. PCR amplification of mouse genomic DNA and human retina cDNA revealed several novel frizzled family members: Mfz2, Mfz3, Mfz4, Mfz6, Mfz7, and Hfz3, and Hfz5, respectively. A mixture of these cloned PCR products were used in a low stringency screen of day 8.5 and day 12.5 mouse embryo cDNA libraries, a day 18.5 embryonic mouse brain library, and a mouse genomic DNA library. Multiple independent cDNA clones were obtained that fell into five classes: Mfz2, Mfz3, Mfz4, Mfz6, and Mfz7. One-half of the Mfz2 coding region was sequenced and was found to be greater than 96% identical at the amino acid level to Rfz2. Mfz2 is therefore likely to represent the orthologue of Rfz2 (Chan et al., 1992). From the Mfz3 and Mfz4 cDNA clones the complete coding region was sequenced (Fig. 1).

Mouse genomic clones encompassing part or all of the coding region were isolated for Mfz2, Mfz3, Mfz4, Mfz6, Mfz7, and Mfz8; partial genomic DNA sequences were obtained from the Mfz3, Mfz4, and Mfz6 clones and these sequences confirm the cDNA and RACE PCR sequences in the regions in which they overlap. The Mfz7 and Mfz8 coding regions were deduced from

![Amino acid sequences of mammalian and nematode frizzled homologues.](image-url)
genomic DNA sequence as no Mfz7 cDNA clones obtained thus far contain the 5’ end of the coding region and Mfz8 cDNA clones have not yet been identified. Conceptual translation of 2.3 and 2.4 kb of contiguous sequence from Mfz7 and Mfz8 genomic clones, respectively, predicts, in each case a single long open reading frame that begins with a putative start sequence and includes all of the characteristic features of a frizzled family member (Fig. 1). Based upon this contiguous homology we tentatively propose that the Mfz7 and Mfz8 genes lack introns within the coding region.

In a parallel screen of an adult human retina cDNA library using PCR products derived from Hfz3 and Hfz5, multiple cDNA clones encoding Hfz3, Hfz5, and Hfz7 were identified. From this collection, the complete sequence of the Hfz5 coding region was determined (Fig. 1). Surprisingly, neither genomic nor cDNA clones were obtained for fz5 orthologues from the mouse library screens described above. However, PCR amplification of mouse genomic DNA using Hfz5-specific primers generated a product, the translated sequence of which is distinct from other mouse frizzled sequences and is 95% identical to Hfz5 over 143 amino acids, indicating that the mouse genome contains a Hfz5 orthologue. This Mfz5 PCR product was used for subsequent RNase protection and in situ hybridization studies (see below).

To examine more fully the diversity of the frizzled family, we searched the NCBI data base of partial cDNA sequences (expressed sequence tags, ESTs) for frizzled homologues. Statistically significant homology was detected in a C. elegans partial cDNA sequence, cm08b2 (21). This cDNA clone was used to identify the corresponding genomic segment from a C. elegans genomic DNA library, and was mapped on the C. elegans genome to yeast artificial chromosomes Y95B8 and Y34D9 on chromosome I. The complete sequence of the mRNA was determined by sequencing this cDNA clone, 5’ RACE PCR products, and the exon regions of the corresponding genomic clone (Fig. 1). These sequences reveal a highly divergent frizzled family member that is encoded by nine exons distributed over 8 kb of genomic DNA (Fig. 2). This gene will be referred to as Cfz1.

As another approach to exploring the diversity of the frizzled family, a set of seven degenerate PCR primers were designed based on an alignment of the sequences of the human and mouse clones described above and the known Drosophila and rat sequences (Table I; YW164-YW285). These, as well as the original pair of degenerate PCR primers were used for PCR amplification of chicken, sea urchin, and zebrafish genomic DNA. PCR products of the predicted size were obtained from a subset of the PCR reactions, and these were subcloned and sequenced. The resulting sequences revealed two, three, and 13 distinct frizzled-like sequences from chicken, sea urchin, and zebrafish, respectively (Fig. 3). The 13 zebrafish PCR products are partially overlapping and define at least 11 distinct genes.

The original pair of degenerate PCR primers, YW157 and YW158, was also used to amplify Drosophila genomic DNA. This PCR reaction was designed to be selective for novel frizzled-like sequences, as this region in the Drosophila frizzled gene is known to be interrupted by a large intron (16). This reaction generated a single PCR product that matched the expected size of a contiguous frizzled coding region. Subsequent work has shown that the corresponding gene, which we will refer to as Dfz2, encodes a frizzled-like protein with less than 35% amino acid identity with the original Drosophila frizzled gene product.

The sequences of the six novel mouse and human frizzled genes reported here brings to eight the number of distinct frizzled-like sequences identified in mammalian genomes. This probably represents an incomplete sampling of frizzled family members because: 1) the PCR reactions selected for family members with high homology to the original group of frizzled sequences used in primer design, 2) PCR amplification on genomic DNA selected for family members that did not contain introns between the primers, and 3) cleavage of PCR products with EcoRI and HindIII followed by preparative gel electrophoresis eliminated products with internal EcoRI or HindIII sites. In support of this idea, whole genome Southern blot analysis (data not shown) indicated that the nematode frizzled gene, Cfz1, contains at least 11 distinct members because: 1) the PCR reactions selected for family members with high homology to the original group of frizzled sequences used in primer design, 2) PCR amplification on genomic DNA selected for family members that did not contain introns between the primers, and 3) cleavage of PCR products with EcoRI and HindIII followed by preparative gel electrophoresis eliminated products with internal EcoRI or HindIII sites. In support of this idea, whole genome Southern blot analysis (data not shown) indicated that the nematode frizzled gene, Cfz1, contains at least 11 distinct members.

3 P. Bhanot, D. Andrew, Y. Wang, and J. Nathans, unpublished data.
hybridization with ZG05, one of the zebrafish PCR products, reveals a large number of hybridizing fragments in chicken, mouse, and human genomic DNA (Fig. 4). As this region shows significant divergence among the cloned frizzled family members at the DNA level, it is likely that some of the hybridizing fragments are derived from frizzled sequences other than the ones reported here.

Implications for Frizzled Structure and Evolution—In Fig. 1, the complete deduced amino acid sequences of Mfz3, Mfz4, Hfz5, Mfz6, Mfz7, Mfz8, and Cfz1 are aligned beneath the sequences of Drosophila frizzled (abbreviated Dfz1), Rfz1, and Rfz2. The lengths of the frizzled proteins range from 525 amino acids (Cfz1) to 709 amino acids (Mfz6). It is apparent from the alignment and from the hydropathy profiles of each frizzled family member (Fig. 5) that there are distinct domains that differ in their degree of amino acid sequence conservation and in their chemical properties. A putative signal peptide is found at the amino termini of all frizzled sequences, consistent with the postulated role of these proteins as integral membrane receptors and with the demonstration that the amino terminus of the Drosophila frizzled protein is extracellular (17). The putative signal peptide region is not conserved at the level of primary sequence. Following the signal peptide, each frizzled protein has a conserved domain of 120 amino acids containing 10 invariant cysteines (Table II), suggesting that this region contains a number of disulfide bonds. This cysteine-rich motif has also been found within a non-helical portion of the mouse α1 chain of type XVIII collagen (37). This is the only sequence in the current GenBank data base that shows statistically significant homology with any part of a frizzled sequence. The cysteine-rich domain is followed by a hydrophilic and highly divergent region that ranges in length from 40 amino acids in Mfz4 to 100 amino acids in Mfz8. These characteristics suggest that this divergent region is minimally ordered and that it may

![Fig. 4. Southern blot hybridization of chicken, mouse, and human genomic DNA probed with the zebrafish frizzled PCR product ZG05.](http://www.jbc.org/)

![Fig. 5. Hydropathy profiles of mammalian and nematode frizzled homologues.](http://www.jbc.org/)

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Frizzled Gene Family

Percent amino acid identity in the cysteine-rich (A) and transmembrane domains (B)
The cysteine-rich and transmembrane regions were defined, respectively, as those regions corresponding to residues 53 to 155 and 246 to 553 in the Drosophila frizzled sequence. For this analysis we have omitted the highly divergent segment of approximately 10 amino acids at the carboxyl terminus of the cysteine-rich region. All pairwise optimal alignments were obtained using GeneWorks software, and the percent amino acid identity was calculated for each alignment with respect to the length of the consensus alignment for that pair. Abbreviations are as in Fig. 1.

| Locus     | Enzyme | Fragment sizes (kb) | M. spretus |
|-----------|--------|---------------------|------------|
| Mfz2-rs1 | PstI   | 4.2, 3.8, 2.2       | 4.6, 3.3, 2.2 |
| Mfz2-rs2 | PstI   | 4.6, 3.8, 2.2       | 4.6, 3.3, 2.2 |
| Mfz3     | Scal   | 11.5, 7.4           | 8.8, 7.6    |
| Mfz4     | BamHI  | 4.1                 | 4.6        |
| Mfz5     | BamHI  | 7.0                 | 8.2        |
| Mfz6     | PvuI   | 2.7                 | 2.0        |
| Mfz7     | TaqI   | 5.6, 0.8            | 4.0, 0.8   |
| Mfz8     | SphI   | –23.0               | 9.5        |

suggest that, like G-protein coupled receptors, the hydrophobic segments of the frizzled proteins form seven membrane spanning α-helices linked by hydrophilic loops (38, 39). In the discussion that follows, we will refer to this part of the protein as the transmembrane region. The transmembrane regions show no primary sequence homology with G-protein coupled receptors, and they lack the most conserved motifs, such as the (Glu/Asp)-Arg-(Tyr/Phe) triplet at the carboxyl terminus of the third transmembrane segment, that are found in nearly all G-protein coupled receptors (40).

Carboxyl-terminal to the transmembrane region, the frizzled proteins show little or no homology. In Drosophila frizzled this region has been shown to reside on the cytosolic face of the plasma membrane, consistent with the extracellular location of the amino terminus and the assignment of seven transmembrane segments based on the hydropathy profile (17). The lengths of the carboxyl-terminal regions range from 25 amino acids in Mfz7 to 200 amino acids in Mfz6.

Chromosomal Locations of frizzled Genes—The present members of the frizzled gene family presumably arose by duplication and divergence from a common ancestral gene. In some instances members of a gene family are clustered at a single locus following their duplication (e.g. the globins). To test this possibility for the frizzled genes, and to begin to look for naturally occurring mouse mutants that may result from frizzled gene defects, the murine chromosomal locations of the frizzled family members (mouse locus designation, fz) were determined using an interspecific backcross mapping panel from crosses of [(C57BL/6j × Mus spretus)F1 X C57BL/6j] mice. This mapping panel has been typed for over 2000 loci that are well distributed among all 19 mouse autosomes and the X chromosome (24). C57BL/6j and M. spretus DNAs were digested with several different restriction enzymes and analyzed by Southern blot hybridization for informative RFLPs using probes specific to each locus (Table III). The strain distribution

serve as an extended linker.

The carboxyl-terminal half of each frizzled protein consists primarily of a 220–250 amino acid region in which seven hydrophobic segments of approximately 20–25 amino acids each are joined by short hydrophilic segments (Figs. 1 and 5). Pairwise comparisons among mammalian frizzled sequences show that the overall amino acid sequence identity in this region ranges from 37% to 85% (Table II). Interestingly, several small stretches of amino acid sequence in this region are highly conserved among all of the frizzled family members (Fig. 1). Although this region in the nematode Cfx1 sequence shows only 13–16% identity in any pairwise comparison, its hydropathy profile closely matches that of the other family members. Among the mammalian and Drosophila frizzled sequences, gaps in the alignment in this region are confined to the hydrophilic segments; alignment of the more divergent Cfx1 sequence requires several small gaps in the hydrophobic segments. These characteristics are reminiscent of those found in sequence comparisons among G-protein coupled receptors, and

| Locus     | Enzyme | Fragment sizes (kb) | M. spretus |
|-----------|--------|---------------------|------------|
| Mfz2-rs1 | PstI   | 4.2, 3.8, 2.2       | 4.6, 3.3, 2.2 |
| Mfz2-rs2 | PstI   | 4.6, 3.8, 2.2       | 4.6, 3.3, 2.2 |
| Mfz3     | Scal   | 11.5, 7.4           | 8.8, 7.6    |
| Mfz4     | BamHI  | 4.1                 | 4.6        |
| Mfz5     | BamHI  | 7.0                 | 8.2        |
| Mfz6     | PvuI   | 2.7                 | 2.0        |
| Mfz7     | TaqI   | 5.6, 0.8            | 4.0, 0.8   |
| Mfz8     | SphI   | –23.0               | 9.5        |

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pattern of each RFLP was then determined for the backcross mice. All backcrosses were to C57BL/6J and, as expected, backcross progeny were either homozygous for the C57BL/6J allele or heterozygous for the C57BL/6J and M. spretus alleles. The simple presence or absence of RFLPs specific for M. spretus was followed in backcross mice. The chromosomal location of each locus was then determined by comparing its strain distribution pattern with the strain distribution patterns for all other loci already mapped in the backcross.

The mapping results are summarized in Fig. 6. Eight loci were detected on seven mouse autosomes. From 130 N2 mice examined, no recombinants were identified that separate Mfz5 and Mfz7, on mouse chromosome 1. Interestingly, Mfz5 and Mfz7 share a degree of similarity that is no greater than that seen in an average pairwise comparison of the available mammalian frizzled genes. The remaining family members are all unlinked. In addition, two loci were detected with a probe derived from Mfz2 (the mouse orthologue of Rfz2): one on chromosome 5 (Mfz2-rs1) and one on 11 (Mfz2-rs2). The human orthologue of Rfz1 has recently been mapped to human chromosome 17q21.1 (41). Mouse chromosome 11 shares a large region of homology with the long arm of human chromosome 17q (Fig. 6; data not shown). The syntenic relationship between these chromosomes and the high degree of homology between Rfz1 and Rfz2 (18) suggests that Mfz2-rs2 is the mouse orthologue of Rfz1 and that the authentic Mfz2 locus (represented by Mfz2-rs1) resides in the proximal region of mouse chromosome 5. The remaining frizzled family members have not been mapped in humans. However, based on synteny, we predict that the human orthologues of Mfz2 will map to human chromosome 7q, Mfz3 to 14q, 13, or 8p, Mfz4 to 11q, Mfz5 to 2q, Mfz6 to 8q, Mfz7 to 2q, and Mfz8 to 10p or 18q (Fig. 6).

Finally, we have compared our interspecific maps with composite mouse linkage maps that report the map location of many uncloned mouse mutations (provided from Mouse Genome Data base, a computerized data base maintained at The Jackson Laboratory, Bar Harbor, ME). The frizzled genes mapped in regions of the composite maps that contain a number of mouse mutations (data not shown). It will be of interest to determine whether any of these mutations are candidates for alterations in a frizzled locus.

Expression Patterns of Mouse Frizzled Genes—As an initial step in exploring the biological roles of the mammalian frizzled proteins, the distribution of transcripts among major organs in the adult mouse was determined for each gene by RNase protection. As seen in Fig. 7, each frizzled gene has a distinct tissue distribution of transcripts, and in all cases transcripts are found in more than one tissue. The most specific distribu-
tions are those of Mfz3, which is expressed at highest levels in the brain, and Mfz5, which is expressed at highest levels in the eye, kidney, and lung. Using the same RNase protection method, Mfz8 transcripts were not detected in any of the nine adult tissues examined.

An anatomic examination at higher resolution was undertaken by in situ hybridization of mouse embryos at days 11.5 and 17.5, of postnatal day 1 animals, and of adult brains. While a detailed account of these experiments is beyond the scope of the present paper, Fig. 8 shows a sample of some of the in situ hybridization patterns for Mfz2, Mfz3, Mfz4, and Mfz5 that suggest in several cases a higher degree of tissue specificity than was indicated by the RNase protection analysis. At embryonic day 17.5, Mfz2 transcripts are most abundant in the ventricular zone in the developing central nervous system, the region that contains actively dividing neuroblasts (Fig. 8C). At all stages tested, Mfz3 transcripts are present at high levels throughout the central nervous system, consistent with the pattern seen by RNase protection (Fig. 8B, E, F, and H). Mfz4, which was observed by RNase protection to be relatively abundant in the adult central nervous systems, is found by in situ hybridization to be present exclusively within the choroid plexus (Fig. 8G); strong Mfz4 hybridization is seen in the lateral ventricles and along the midline in the third ventricle. At embryonic day 17.5 and postnatal day 1, Mfz5 transcripts are most abundant in the developing retina and/or the underlying choroid, consistent with the strong signal obtained with eye RNA by RNase protection (Fig. 8, A and D).

**DISCUSSION**

The experiments reported here reveal a large family of putative transmembrane receptors that are related to the Drosophila tissue polarity gene frizzled. The identification of eight frizzled genes in mammals and at least 11 in zebrafish indicates that members of this family are likely to play multiple roles in vertebrate development and/or physiology. The finding of frizzled family members in organisms as diverse as humans and nematodes indicates that the frizzled proteins are likely to be part of a universal signaling system in the animal kingdom. Moreover, the expression of frizzled family members in many different tissues and during embryonic development suggests that they are involved in a wide variety of developmental and/or homeostatic processes. We consider below the structures and possible functions of the frizzled proteins in light of our current knowledge regarding Drosophila frizzled.

The aberrant orientation of surface structures seen in Dro-
sophia frizzled mutants indicates that frizzled is involved in transmitting or interpreting a signal that determines the polarity of the bristle and hair cell precursors. Interestingly, in weak frizzled alleles hairs remain locally aligned but show global disorganization, resulting in whorls composed of several dozens of hairs. As discussed in Adler et al. (13), one interpretation of this observation is that frizzled receives distinct global and local polarity signals, with receipt of the latter signal being more robust. Alternately, frizzled may be involved in the maintenance of a single external polarity signal which becomes globally disorganized but remains locally intact when frizzled activity is diminished. The latter model would also account for the observed local spread of the altered tissue polarity phenotype in genetic mosaics. Although these models differ in the hypothesized role for frizzled, they both postulate the existence of an extracellular ligand-receptor system that must ultimately organize the cytoskeleton of the hair and bristle cells to direct their growth.

The frizzled sequences reported in Vinson et al. (14), Chan et al. (18), and the present work bring to 10 the number of full-length frizzled sequences known. All frizzled sequences share the same overall domain structure. Sequence comparisons show that the cysteine-rich domains and the transmembrane domains are strongly conserved, although in the case of Cz21 the conservation in the transmembrane domain is principally at the level of hydrophyt. These sequence analyses suggest a working model in which a cysteine-rich extracellular domain is tethered by a variable linker region to a bundle of seven membrane spanning α-helices (Fig. 9). If it is assumed for this discussion that frizzled proteins act as receptors, then this structural model suggests a dynamic model of transmembrane signal transduction analogous to that proposed for the subfamily of G-protein coupled receptors with amino-terminal ligand binding domains (42, 43). Specifically, we envision frizzled signal transduction to proceed through the following steps: 1) extracellular ligand binds to the cysteine-rich domain, 2) ligand binding changes the interaction of the cysteine-rich region with the transmembrane domain (possibly as a result of direct contact between the ligand and the transmembrane domains), 3) changes in this interaction on the extracellular face of the protein result in a rearrangement of transmembrane α-helices, and 4) rearrangement of transmembrane helices alters the cytosolic face of the protein which either promotes or inhibits interactions with second messenger components. Elaborations or variations on this model are readily envisioned, including the possibility that in vivo the frizzled proteins function not as monomers but as part of a homor heteromultimeric complex.

If frizzled proteins act as receptors, that would imply the existence of a corresponding family of ligands. In the simplest case, if there is one ligand for each receptor, then mammals would be expected to have at least eight frizzled ligands, and zebrafish at least 11. Currently, the only family of ligands known to be of this size and for which no receptors have been identified are the Wnt proteins (10). The availability of a large number of frizzled genes should facilitate a biochemical test of the possibility that these two families of proteins interact directly. Finally, the lack of primary sequence homology between the frizzled family and known receptor families suggests that these proteins may couple to a novel effector pathway. The existence of multiple frizzled proteins implies either the existence of a large number of homologous effector pathways or a convergence on a smaller number of pathways, as seen in G-protein coupled signal transduction.

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A Large Family of Putative Transmembrane Receptors Homologous to the Product of the *Drosophila* Tissue Polarity Gene *frizzled*

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