[Frontiers in Bioscience 11, 2442-2464, September 1, 2006]

WNT/Frizzled signaling in eye development and disease

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1. ABSTRACT

The canonical Wnt/Fzd signaling pathway is highly conserved among various species. Increasing evidence is accumulating for non-canonical Wnt signaling pathways, analogous to those discovered in *Drosophila,* being operative in vertebrates. Similarly, the networks of genes involved in eye development show significant conservation during evolution. The amenability of *Drosophila* for genetic manipulation and analysis of ocular phenotypes has delivered a great deal of information about the roles of the Wnt/Fzd signaling pathways at various stages of ocular development and growth, particularly in regulating the formation and size of the eye field, cell proliferation, polarity and differentiation. In addition to the numerous recent studies that have identified the expression of various components of these signaling pathways in the developing vertebrate eye, functional studies have revealed significant parallels in the way that Wnt/Fz signals regulate the formation of the vertebrate eye field and also the proliferation and differentiation of cells, particularly in the lens and retina. Significant advances have also recently been made in identifying mutations in these signaling pathways that underlie or contribute to various ocular diseases such as exudative vitreoretinopathy, retinal degenerations, cataract, ocular tumors and various congenital ocular malformations. Combined with the mechanistic studies in vertebrate and invertebrate models, these studies point to important functional roles for Wnt/Fzd pathways in the human eye. Further investigation of how these pathways function during eye development and growth may yield important insights into novel therapeutic approaches to treat or prevent diseases that cause blindness.

2. INTRODUCTION

Over a century of genetic experimentation has made *Drosophila melanogaster* an excellent model organism for the genetic dissection of the signaling pathways that regulate metazoan development. The Wnt signaling pathway has been extensively analyzed in *Drosophila* and many genes implicated in Wnt signaling were first identified in this organism. In fact, the term "Wnt" is a concatenation of the *Drosophila* "w*ingless (wg)*" and murine "*Int-1*" orthologous secreted signaling proteins (1). Since the characterization of the *Drosophila wingless* (2, 3) and *frizzled* (4, 5) mutants 20 to 30 years ago and the subsequent identification of numerous vertebrate *Wnt* and *Frizzled* (Fz/Fzd) homologues, there has been an explosion of interest in the roles of these signaling molecules in development and disease. Here we have reviewed what is known about Wnt/Fzd signaling in eye development, firstly in *Drosophila* and subsequently in vertebrates (fish, amphibians and mammals). Increasingly, evidence is surfacing that mutations in genes involved in Wnt signaling cascades underlie human diseases and towards the end of this article we review the progress in the identification of these mutations in various human ocular diseases.

Wnts comprise a large family of secreted glycoproteins of which 19 different genes have been identified in mice and humans and seven have been identified in the *Drosophila* genome (*wg*, *DWnt2, DWnt3/5, DWnt4, DWnt6, DWnt8/WntD and DWnt10*; see http://www.stanford.edu/~rnusse/wntwindow.html (1) for most current information). These signaling molecules participate in a wide range of events during embryonic development and are also implicated in modulating cellular functions in adult tissues (6). Wnts bind to the Frizzled (Fz/Fzd) family of serpentine receptors (ten known vertebrate and four *Drosophila* members), which have seven transmembrane domains and can activate at least three different intracellular signaling pathways; Wnt/beta-catenin, Wnt/planar cell polarity or Wnt/Ca^{2+} pathways (see other reviews in this issue). The Wnts have tentatively been grouped into two classes: the Wnt1 class (Wnt1, 3, 3a and 8) predominantly activate the canonical signaling pathway and affect cell fate and are active in axis duplication assays in the early amphibian embryo; the Wnt5a class (Wnt4, 5a and 11) predominantly activate non-canonical signals and are generally inactive in axis duplication assays (7). However, some Wnts may activate both pathways under certain circumstances (e.g. Wnt5a) and thus the pathway activated by a particular Wnt may depend on the Fz receptors expressed and also on the context and environment of the cell receiving the signal.

3. CANONICAL WNT SIGNALING

The canonical Wnt/beta-catenin pathway is the best characterized and understood Wnt signaling pathway (6, 8) and its conservation across 600 million years of metazoan evolution illustrates the importance of this pathway in the regulation of animal development.

In *Drosophila*, as with vertebrates (see below) a co-receptor Arrow (LRP5/6 ortholog) is required to act with the Frizzled (Fz) receptor for signaling via the canonical pathway. Only a single Dishevelled protein is present in *Drosophila,* which acts to inhibit the glycogen synthase kinase-3beta (GSK-3) ortholog, Zw3/Shaggy, from phosphorylating Armadillo (beta-catenin). A single Axin protein co-operates with one of two adenomatous polyposis coli (APC) proteins to regulate Armadillo phosphorylation. The APC proteins appear to be redundant in many tissues. The core proteins of the Wnt canonical signaling pathway are thus highly conserved, with differences observed in the numbers of paralogs observed within species. Vertebrates also appear to have evolved novel secreted inhibitors of Wnt signaling (Frp, WIF, Dkk, Kremen, Wise) that are not present in *Drosophila* (6, 9).

In the inactive state (i.e. absence of Wnt ligands), cytoplasmic beta-catenin, which is also involved structurally in linking cadherins to the actin cytoskeleton in adherens junctions, is recruited to a degradation complex consisting of axin, APC and GSK3beta. Phosphorylation of beta-catenin by GSK3beta and also casein kinase-1, results in its ubiquitylation and targeting for proteasomal degradation. When Wnt ligands bind to the Fzd receptors they form a complex with the single-pass transmembrane LDL-receptor-related proteins 5 or 6 (LRP5, LRP6), which appear to be required for activation of the canonical pathway (10-12). Activation of the Fzd receptor leads to the phosphorylation of Dishevelled (Dsh) proteins and their recruitment to the membrane. Active Dsh leads to the recruitment of the beta-catenin destruction complex to the membrane where axin binds to Dsh, via their respective DIX (Dishevelled and Axin) domains, and to the cytoplasmic tail of LRP5/6, resulting in axin degradation. Dsh also inhibits GSK3beta activity. The degradation of axin and inhibition of GSK3beta leads to increased stability of beta-catenin in the cytoplasm and, as levels rise, it becomes translocated to the nucleus, where it interacts with lymphoid enhancer factor/T-cell factor (LEF/TCF) transcription factors to activate transcription of Wnt target genes.

4. NON-CANONICAL WNT SIGNALING

The non-canonical Wnt pathways diverge from the canonical pathway downstream of Dsh and appear to overlap with each other. The planar cell polarity (PCP) pathway has been best characterized in *Drosophila* where it regulates the alignment of cells within a planar epithelium as exemplified by the alignment of the wing hairs (trichomes) or the ommatidia of the eye (13-16). Recent evidence also implicates the PCP pathway in convergent extension movements, typified by extension of the body axis and intercalation of cells to form the notochord during *Xenopus* and zebrafish gastrulation, and in the alignment of the sensory epithelium in the murine inner ear. In *Drosophila*, the PCP pathway involves several key proteins (Fz, Dsh, Rho, Rho Kinase, Flamingo, Strabismus, Prickle), which are involved in cytoskeletal rearrangements that result in the asymmetric distribution of these proteins on different sides (proximal or distal) of the cell in response to

a polarizing signal. Proteins such as Fz, Dsh and Rho become localized to the distal membrane whereas Strabismus and Prickle become localized to the proximal membrane. During activation of this pathway Dsh becomes localized to the membrane via its DEP (Dishevelled, egl-10, and pleckstrin) domain (17), where it interacts with Daam 1, which interacts with the small GTPase, Rho, resulting in activation of the Rho-associated kinase (ROK). The small GTPases Rho, Rac and Cdc42 have all been implicated in PCP pathway signaling and presumably are the effectors of cytoskeletal changes. However, Rac, Rho and the DEP domain of Dsh have also been shown to cause activation of the c-Jun N-terminal kinase (JNK) pathway. There is also evidence that non-canonical signaling can result in calcium fluxes within the cell and activation of calcium-sensitive protein kinase C (PKC) and calmodulin-dependent kinase II (15, 16).

5. MODULATORS OF WNT/FZD SIGNALING

Wnt signaling can be modulated at various levels. Extracellular modulators of Wnt binding to Fzd receptors can be broadly divided into two classes; the secreted frizzled-related protein (Sfrp) and Dickkopf (Dkk) classes (7). The Sfrp proteins, which have homology to the extracellular cysteine-rich domain of Fzd receptors but lack the transmembrane domain, bind directly to the Wnt ligand and thus alter their ability to bind and activate Fzd receptors. The Dkk family of proteins, of which Dkk1 is the most studied, acts predominantly by binding the Wnt co-receptors LRP5/6 and thus act to antagonize the canonical signaling pathway. However, recent evidence also implicates Dkk1 in activation of non-canonical pathways (15) and, in the case of Dkk2, activation of canonical signaling when expressed ectopically (18).

Wnt signals may also be modulated in the cytoplasm by factors that affect the phosphorylation of beta-catenin and the activity of the beta-catenin destruction complex. Within the nucleus, there are factors that affect the activity of the Lef/Tcf-beta-catenin complex, the shuttling of beta-catenin between the nucleus and the cytoplasm (19) as well as a number of proteins that interact with beta-catenin (6).

6. DROSOPHILA EYE DEVELOPMENT

The *Drosophila* eye has been a favored tissue for the analysis of many developmental signaling pathways for two main reasons. The adult insect compound eye is a reiterative structure composed of many individual eye units or ommatidia arranged in a precisely ordered hexagonal array (figure 1A). This provides a structure where patterning defects can be easily discerned by external observation of disruption of the array (20). The second reason for its importance as a model is that the eye is an organ that is not essential for viability or fertility. Therefore the eye can be disrupted or completely ablated by genetic mutation without affecting viability of the mutant strain. The development of eye-specific promoters for transgene analysis and tissue-specific gene ablation has meant that signaling pathways, which are required for multiple aspects of development of the organism, can be assayed without the complication of organism lethality (21).

6.1. Generation of the optic primordium

Tissues that will develop into the adult eye and antennal structures derive from two bilateral groups of approximately 20 cells that arise late in embryogenesis within the optic primordium of the embryonic blastoderm (22, 23). These cells invaginate to produce an epithelial flattened sac, the primordial eye-antennal imaginal disc. The *Pax6* family members, *eyeless* and *twin of eyeless*, play a key role in specification of the eye primordia and both are expressed within the cells that generate the eye-antennal disc (24-26). After completion of embryogenesis, *Drosophila* larvae undergo three molts prior to metamorphosis. The eye-antennal disc continues to proliferate throughout the three larval stages, or instars, until it contains approximately 2000 cells in the third instar larva. It is during the third instar stage that the disc initiates the differentiation program that leads to formation of the adult eye structure.

6.2. The structure of the adult Drosophila eye

The adult eye consists of approximately 750 ommatidia that are each composed of 8 photoreceptor neurons (named R1-R8) and 11 accessory cells (27). The accessory cells consist of four lens-secreting cone cells, two primary pigment cells and shared secondary and tertiary pigment cells. The pigment cells serve to optically insulate each ommatidium from its neighbors. Light-responsive opsin molecules are present in apical microvillar extensions, or rhabdomeres, of the elongated photoreceptor neurons (28). Rhabdomere position and morphology facilitates photoreceptor identification as they are arranged in a stereotypical trapezoidal array within the centre of each ommatidium (figure 1B). R1-R6 also have larger rhabdomeres that form the trapezoid with a smaller R7 rhabdomere in the centre. The R8 rhabdomere is located below R7 such that only 7 rhabdomeres are visible in tangential sections of the adult retina (27). Photoreceptors are also functionally distinct with respect to their spectral sensitivity. The R7 rhabdomere contains an ultraviolet-sensitive opsin of either the Rh3 or Rh4 type (29, 30), R8 carries either an Rh5 or Rh6 blue-green-sensitive opsin (31, 32) and the outer R1-R6 photoreceptors carry an R1 blue-sensitive opsin (33).

6.3. Differentiation of the adult eye

The epithelial monolayer that comprises the eye-antennal imaginal disc proliferates during the first two larval stages without differentiating. The eye field is defined at the end of the second larval instar by the activity of a *Pax6* family-induced transcriptional mechanism. Differentiation of the eye is initiated in a progressive manner during the third larval instar, when a morphological indentation, the morphogenetic furrow, is formed in the epithelium at the posterior edge of the eye field (figure 1C). The furrow results from the integrated activities of a number of signaling pathways causing a constriction of apical rings of actin within the columnar epithelial cells (34-37). Anterior movement of the morphogenetic furrow across the plane of the disc epithelium results in the establishment of a column of ommatidial founder cells

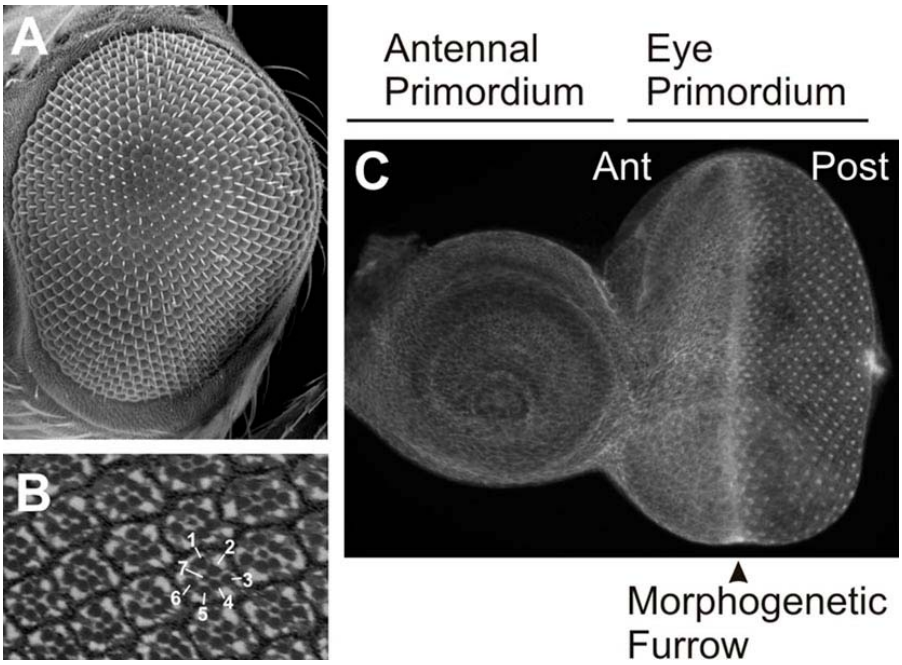

Figure 1. The *Drosophila* compound eye. A. A scanning electron micrograph of an adult compound eye shows the individual facets, or ommatidia. B. Each ommatidium contains eight photoreceptor neurons. The rhabdomeres of photoreceptor neurons are visible as darkly stained dots in a section through the retina of an adult eye. The R7/R8 rhabdomere (7) of each ommatidium is smaller than and central to the outer rhabdomeres (1-6) that form a trapezoidal array in each ommatidium. C. An eye-antennal disc from a third instar larva stained to illustrate cell membranes. Ommatidial clusters can be seen differentiating posterior (Post) to the morphogenetic furrow (Ant, anterior).

approximately every two hours (20, 27). Interestingly, a progressive differentiation of the retina may not be unique to arthropods as zebrafish (38, 39) and mammalian (see below) retinal differentiation is initiated in the centre of the eye field and progresses in a radial wave towards the periphery. Signal feedback relays result in continuous generation of new ommatidial founders and progression of the morphogenetic furrow such that at a single time-point the youngest ommatidia are found in the most anterior region of the eye field that has been traversed by the furrow (40, 41).

The first cell in each ommatidial precursor to be specified is the R8 photoreceptor (42). The R8 cell then recruits other photoreceptor cells to the forming ommatidial precluster via sequential specification. R2 and R5 specification is followed by R3 and R4. After formation of the 5 cell precluster all uncommitted cells undergo mitotic division, followed by recruitment of R1, R6, and finally R7. Recruitment of all 8 photoreceptors occurs during the third larval instar stage. The last components of the ommatidia to be recruited, while the animal remains a larva, are the four cone cells. The rest of the accessory cells of the ommatidia are recruited during the pupal stage that is characteristic of homometabolous metamorphosis. Extra pigment cells are often incorporated into developing ommatidia during pupal differentiation. These cells are eliminated by apoptosis to form the precise ommatidial lattice. The boundaries of the adult eye are also shaped by the apoptosis of entire ommatidia (27).

7. WINGLESS/FZ SIGNALING AND *DROSOPHILA* EYE DEVELOPMENT

The Wg signaling pathway has been shown to be required at multiple points in development of the *Drosophila* eye, from specification of the eye field, through to differentiation of the retina and determination of retinal polarity.

7.1. Wingless limits the size of the Drosophila eye field and regulates retinal cell proliferation

The eye-imaginal disc is a primordium of the adult eye but also produces the antenna and much of the adult head capsule. Inhibition of the Wg pathway is crucial for specifying the presumptive eye field from the head capsule. The eye field generates the retina and is specified by the action of *eyeless* and *twin of eyeless* (24, 26), along with downstream transcription factors *eyes absent*, *sine oculis* and *dachshund* (43-45). It is only within this field, specified by the activity of *Pax6* genes, that the morphogenetic furrow initiates photoreceptor development.

Wg is required for many developmental processes that occur during embryonic and larval stages. Animals lacking Wg activity, arrest with altered morphology during embryogenesis (46-48). Royet and Finkelstein (49) utilized a temperature-sensitive allele of *wg* to demonstrate that *wg* is required for patterning of lateral and mediolateral head regions that separate the developing bilateral eyes. Animals of this genotype develop normally when raised at 17°C but

if shifted to the restrictive temperature of 25°C during the third instar period adults fail to eclose and exhibit loss of lateral and mediolateral head regions. A subsequent study (50) showed that *wg* expression in the eye field is suppressed by activity of the bone morphogenetic protein (BMP) family molecule, Decapentaplegic (Dpp). Loss of *Dpp,* and hence expression of *Wg,* within the eye field leads to loss of eye structures and development of head cuticle within the eye primordium. In order to determine if *wg* directly suppressed eye development, the Wg pathway was activated by inducing clones in third instar eye discs, which were mutant for *Zw3/Shaggy*. Cells in these clones were unable to phosphorylate and degrade Armadillo and hence exhibited unregulated Wg pathway activation. The clones expressed the Wg target *orthodenticle* and formed adult head cuticle instead of eye tissue.

The eye primordium becomes established during the early second instar larval period with the onset of *eyes absent* (*Eya*) expression. The eventual size of the eye field is ultimately the result of a complicated interaction between Notch signaling, which induces proliferation of eye field cells, and patterning by the Dpp and Wg pathways (51). *Wg* is normally expressed throughout the first instar eye field where it probably plays a role in initial specification of anterior (or undifferentiated) identity as ectopic induction of Wg signaling in differentiating retinal cells results in ectopic expression of *eyeless*. These *eyeless*-expressing cells fail to differentiate but proliferate extensively. This requirement for Wg appears to be transient as in third instar discs, *eyeless* expression in the undifferentiated cells anterior to the morphogenetic furrow is not dependent on Wg signaling (52). *Wg* has, however, been shown to suppress expression of the *Eyeless* effectors *eyes absent, dachshund* and *sine oculis* anterior to the morphogenetic furrow indicating that it also functions to limit the final size of the eye primordium. The normal expression pattern of *wg* in the third instar eye disc is complementary to the expression of *eyes absent* and *sine oculis* suggesting that the Wg pathway signals cells to initiate formation of a boundary between presumptive head and eye fields (53). The ectopic non-eye tissue formed due to ectopic Wg activity also exhibits a variety of inappropriate developmental fates such as expression of *distal-less,* a gene associated with specification of legs, wings and antennae. Ectopic Wg thus appears to result in a transdetermination of eye field cells to other developmental fates (53). It has been previously observed that ectopic expression of *eyeless* in other organ primordia, e.g. wing imaginal discs, can induce ectopic eyes. However, these extra eyes only form in regions of the disc that do not express *wg*, again suggesting that *wg* acts to restrict the size of the boundary of the eye field by suppression of the transcription factors *eya*, *sine oculis* and *dachshund* downstream of *Pax6* family genes (25).

7.2. Wingless regulates progression of the morphogenetic furrow

Retinal differentiation is initiated by the passage of the morphogenetic furrow across the eye field. The furrow resembles a moving compartment boundary analogous to the anterior-posterior (A/P) boundary in developing leg and wing imaginal discs. In each of these discs the Hedgehog signaling molecule diffuses from the posterior region of the disc to induce expression of *dpp* at the A/P boundary (54). Likewise in the eye disc, *hedgehog* is expressed posterior to the morphogenetic furrow and induces *dpp* in a region that overlaps the furrow (36, 50, 55). The morphogenetic furrow therefore has an organizer capacity and yet it is not strictly a compartment boundary as cells on either side of the furrow are not lineage restricted.

Hedgehog and Dpp initiate propagation of the furrow at the posterior margin of the eye disc. Propagation of the furrow is regulated by a complex gene activation loop that has not been clearly defined although it appears that *hedgehog* induces *dpp* and Ras pathway activation in the furrow, which together activates expression of the proneural transcription factor, *atonal*. Atonal heterodimerizes with the ubiquitously expressed transcription factor, Daughterless, to induce specification of R8 photoreceptors. The R8 cells act as founders of individual ommatidial clusters. Daughterless is also required to activate expression of *hedgehog* and thereby initiate furrow progression (56, 57).

Wg plays an important role in restricting morphogenetic furrow initiation to the posterior margin of the eye disc as loss of *wg* causes ectopic furrows to form at the dorsal and ventral margins (58, 59). High levels of Dpp present at the posterior margin repress Wg activation, thereby restricting Wg activity to dorsal and ventral edges (60). Wg not only plays a role in prevention of furrow formation but also influences furrow movement as ectopic activation of Wg prevents furrow progression (52). The ability of *wg* to block furrow progression can be suppressed by co-expression of activated Ras but not by co-expression of *dpp*. This suggests that Wg normally acts downstream of Dpp but upstream of Ras to prevent furrow initiation and movement (61). The mechanism by which Wg prevents furrow initiation appears to be by direct repression of *daughterless (da)* expression (56). *Wg* is normally expressed in a region of the eye disc that is lacking in *da* and ectopic *wg* represses *da* expression. The ability of *wg* to prevent furrow formation can also be suppressed by co-expression of *da* from a heterologous promoter, indicating that the suppression is due to a direct interaction between Wg signaling and the *da* promoter (56).

7.3. Wingless regulates dorsoventral polarity of the developing eye

Ommatidia in the dorsal and ventral halves of the adult eye display mirror-image symmetry centered about the equator, or dorsoventral midline. This opposing chirality is formed as cells are recruited into the developing ommatidia. As the ommatidia develop, they rotate though 90° in opposing directions in the dorsal and ventral compartments (20). Dorsoventral polarity within the eye disc is established well before ommatidia begin to differentiate and is also influenced by Wg/Fz signaling (62).

Ommatidial rotation appears to be dependent on both canonical and non-canonical Fz signaling pathways. The dorsal compartment of the eye disc is determined by expression of *pannier*, a member of the GATA family of transcription factors that induces *wg* expression (63). *Wg* in turn induces expression of the *Iroquois* complex (*Iro-C*) of homeobox genes in the dorsal compartment (64, 65). The Iro-C homeodomain proteins restrict expression of the Notch modulator, *fringe* (66), to the ventral compartment of the eye disc, ultimately leading to activation of Notch specifically along the equator (52). A secondary signal propagated from the equator then polarizes the eye disc (62, 67). While the nature of this signal is undetermined it has become clear that *Fz* is required to mediate correct ommatidial rotation (68). Drosophila Fz is a member of the family of proteins that can act as Wg/Wnt receptors (69), but as yet there is no direct evidence that a Wnt signal emanates from the equator to polarize the disc. What is clear, however, is that this secondary Fz signal does not involve the canonical Wnt pathway to induce polarization of ommatidia but involves Fz interaction with a protein complex, including the central components of the PCP pathway Flamingo, a cadherin-like molecule, and Strabismus (70-72). The ability of Fz receptor to activate this PCP pathway appears to be reliant on their localization in the apical plasma membrane; *Drosophila* Fz1 is predominantly localized in the apical membrane, associated with junctional complexes, whereas *Drosophila* Fz2 is localized throughout the apico-basal membrane. This differential localization is mediated by sequences in the cytoplasmic tails of the Fz receptors as experiments using chimeric *Drosophila* Fz1/Fz2 constructs indicate that the 61 amino acid extension of the Fz2 carboxy terminal inhibits the apical localization of Fz receptors. Assays of ommatidial polarity in *Drosophila e*xpressing such chimeric receptors in the developing eye, reveal that not only is apical localization important for PCP signaling, but the seven-span transmembrane domain of Fz1 is required for this signaling to be effective (73).

7.4. Wingless signaling shapes the pupal eye

The patterning events that occur in the third instar eye disc are further refined during pupariation and Wg signaling plays several roles that lead to final formation of the adult eye. Several rounds of apoptosis occur during eye development to eliminate excess and undifferentiated cells (27). Wg appears to be secreted from cone cells early in pupal development to induce early-stage apoptosis of excess inter-ommatidial cells (74).

As the adult eye nears final development, the periphery of the eye differentiates itself from the rest of the ommatidial array. The mechanosensory bristles that cover the surface of the eye do not form in the periphery of the eye (27) and work by Cadigan and colleagues (56, 75), suggest that Wg signaling also regulates this alteration in patterning. Early experiments indicated that expression of ectopic *wg* across the developing eye eliminates bristle formation. This suggested that loss of *wg* expression in the periphery would cause the reverse phenotype, or formation of bristles at the margin. This was observed not to be the case although loss of the signal transduction pathway by removal of Armadillo did cause ectopic bristle formation. Ectopic bristles could also be induced 30% of the time in mutant clones that lacked *wg*, *DWnt4, DWnt6* and *DWnt10* suggesting that another member of the Wnt pathway or redundant Wnt factors regulate bristle formation at the periphery of the eye (56, 75).

Wg is also known to play one more role in differentiation of the eye periphery. A narrow dorsal region of the fly retina is specialized for the detection of polarized light. Ommatidia of the dorsal rim have specialized central R7 and R8 rhabdomeres that are larger than normal and contain the Rh3 opsin. The development of these specialized ommatidia is induced by *wg* expression in the adjacent presumptive head cuticle. By experimentally varying the levels of Wg in the developing dorsal eye Tomlinson (76) was able to demonstrate that high levels of signaling immediately adjacent to the source of *wg* in the head capsule induces formation of a pigmented rim devoid of ommatidia that surrounds the eye, and intermediate levels of *wg* induce the formation of the specialized dorsal rim ommatidia inside the pigment rim. Although *wg* is also expressed at the ventral margin, the specialized ommatidia only form at the dorsal margin due to dorsal-specific expression of the Iro-C homeodomain proteins. *Wg* together with the Iro-C proteins can induce expression of another homeodomain protein, Homothorax, which was shown to be sufficient to promote formation of dorsal rim ommatidia (76, 77).

Wnt signaling plays multiple roles in development of the eye from initiation of the eye field through to final differentiation of the adult eye. There will almost certainly be more roles identified for this family of signaling molecules in eye development as new alleles of members of the signal transduction pathway are isolated.

8. VERTEBRATE EYE DEVELOPMENT

The genetic network of transcription factors involved in eye development displays significant evolutionary conservation. Many of the genes involved in *Drosophila* eye development (*Ey, Eya, Dac, Homothorax, Dpp, Sine oculis* etc) have vertebrate orthologs that are expressed and have important roles in the developing vertebrate eye. In particular, *Pax6* is a highly conserved transcription factor that appears to sit at the apex of this network. Based on the fact that *Pax6* mutations result in disrupted eye development in flies and vertebrates as well as its ability to induce ectopic eyes in flies and *Xenopus*, have led to the proposal that *Pax6* is the “Master Eye” gene (78, 79). However, there is also evidence for divergence and intercalary evolution, whereby genes within this network have been duplicated and redeployed for different or even redundant functions. As the eye has evolved into a range of organs with varying complexity, ranging from the relatively simple eye of *Cnidaria* and *Planaria*, through to the compound eye of insects and the ‘camera-type’ eye of vertebrates, similar sets of genes or pathways have been redeployed to generate different eye structures (80-82). This structural diversity has involved recruitment of additional genes (eg. lens crystallins) but also changes in the regulation of existing genes and pathways. For instance,

the interdependence of expression of several key eye genes (Pax6, Six3, Dach1, Eya1) is different between flies and mice (83). Of interest for this review is that signaling pathways used during eye specification and induction (Shh, Wnt, FGF, BMP,) appear to be re-used during differentiation of ocular tissues such as the lens and retina. The following is a brief review of vertebrate eye development to place into context the current knowledge about the role of Wnt/Fz signals during this process. For more detailed information, readers are directed to several extensive recent reviews of vertebrate eye development (84-91).

The earliest molecular events involved in development of the vertebrate eye occur during late gastrulation and the subsequent specification of the anterior neural plate (ANP). The classic experiments by Mangold and Spemann (92) showed that during gastrulation signals from the 'head inducer' of the organizer have the capacity to induce secondary head structures. Numerous studies have since shown that complex interactions of secreted molecules from the organizer with Wnt, transforming growth factor-beta (TGFbeta) superfamily, fibroblast growth factors (FGF) and insulin-like growth factor (IGF) signaling pathways specify the antero-posterior and dorso-ventral axis of the embryo (93, 94). A major consequence of these inductive interactions is the formation of the neural plate and the specification of a region of cells within the ANP as presumptive retina. A region of ectoderm lateral to the neural plate and adjacent to the presumptive retinal cells is fated to give rise to the lens (89). The regional patterning of the ANP involves complex interactions between FGF, BMP, retinoic acid, hedgehog and Wnt signaling pathways (95). Recent data indicates that a localized gradient of Wnt signals within the ectoderm of the ANP plays key roles in specifying cell fates; low levels of Wnt/beta-catenin signaling promote rostral (telencephalon and eyes) fates whereas high signaling activity specifies more caudal (diencephalons) fates (95).

The eye-forming region is characterized by the overlapping expression of several homeodomain-containing genes (84, 88-90, 96), including *Otx2, Rx, Pax6, Sox2, Lhx2, Six3* and *Six6/Optx2*, all of which appear individually to be required for eye formation (96). Initially, the eye-forming region of the neural plate is a broad, crescent-shaped domain in the ANP. Subsequent signals (including sonic hedgehog, (Shh)) from the midline prechordal plate result in the formation of increasingly restricted bilateral eye fields (97, 98).

During and following neurulation, the forebrain neural ectoderm, containing the retinal eye field, evaginates to form the optic vesicles, which grow laterally to contact and associate intimately with the presumptive lens ectoderm (figure 2). Reciprocal inductive interactions between the optic vesicle and the overlying ectoderm result in the localized thickening of both epithelia to form the retinal disc and lens placode (99). The lens placode and retinal disc subsequently invaginate to form the lens pit and a bilayered optic cup; the inner layer will form the neural retina and the outer layer forms the pigmented epithelium of the retina. Further invagination and closure of the lens pit gives rise to the lens vesicle, which detaches from the optic cup (figure 2). The lens in turn secretes morphogens that have an inductive role in corneal development (100). However, the nature of these secreted signals is still not known (100, 101).

The space between the lens vesicle and optic cup becomes infiltrated with blood vessels that arise from the hyaloid artery, which penetrates the optic cup via the optic fissure of the optic stalk (102). The hyaloid artery traverses the primitive vitreous and forms a network of vessels across the posterior surface of the lens, the tunica vasculosa lentis (TVL). The TVL extends anteriorly to cover the anterior surface of the lens capsule to form the pupillary membrane (PM) and this extensive network of blood vessels functions to provide nutrients for the developing lens and contributes to the formation of the vitreous. Postnatally, the hyaloid vasculature and the PM/TVL regress via macrophage-mediated apoptosis (103, 104).

The retina, like the lens, is supplied during fetal development by the hyaloid artery and its branches at the optic nerve head (vasa hyaloidea propria), which spread as a network of vessels in the vitreous anterior to the retina. These intraocular vessels subsequently degenerate by apoptosis at birth or soon after (103). Coincident with this regression of the fetal vasculature is the development of the retinal vasculature originating from the optic nerve head and spreading as dense network over the inner retinal surface of the retina (105). This network forms in association with a network of astrocytes that express vascular endothelial growth factor (VEGF), which regulates the formation of the vasculature (106, 107). After establishment of this primary inner retinal vascular network, vessels start to sprout downward, into the outer and inner plexiform layers, where they establish secondary vascular networks parallel to the first (108). The secondary networks appear not to be associated with retinal astrocytes, but has been hypothesized to arise from a metabolic hypoxia-induced expression of VEGF by the Muller glia (109). It is generally considered that the primary vascular development occurs by vasculogenesis, whereas the secondary network in the inner plexiform layer arises by angiogenesis. However, this may vary according to species (particularly human versus mouse) and is still an area of controversy (110, 111).

8.1. Lens induction and differentiation

The first morphological sign of lens formation is the appearance of the lens placode (figure 2), which is followed soon after by the expression of the lens specific protein, alpha-crystallin, at the lens pit stage (112). The signaling pathways involved in lens induction include FGFs and BMPs and various transcription factors such as the Meis transcription factors (orthologs of *Homothorax* in *Drosophila*), Pax6, Six3 and FoxE3 (87).

Within the lens vesicle, the posterior cells that face the optic cup become increasingly elongated and differentiate into the primary lens fiber cells as evidenced by the expression of fiber-specific proteins (e.g. beta- and

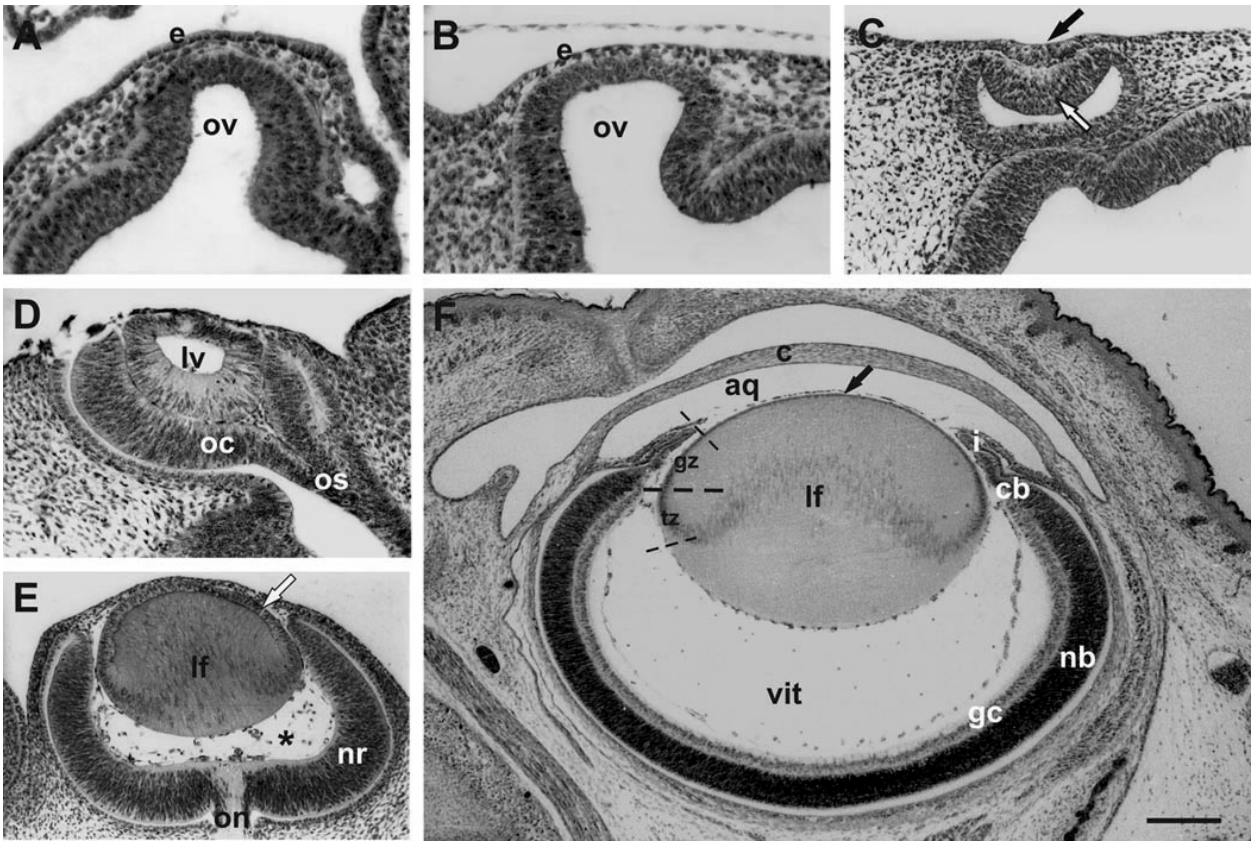

Figure 2. Development of the vertebrate 'camera' eye.A. The optic vesicle (ov) evaginates towards the overlying lens ectoderm (e). The tissues come into very close contact (B), permitting reciprocal interactions that result in (C) local thickening and formation of a lens placode (black arrow) and retinal disc (white arrow). D. The lens placode and retinal disc invaginate and form a lens vesicle (lv) and optic cup (oc), respectively. The optic cup retains contact with the forebrain via the optic stalk (os). E. The anterior cells in the lens vesicle form the lens epithelium (white arrow), whereas the posterior cells elongate and form the lens fibers (lf). At the same time, the optic cup detaches from the lens to form the vitreous chamber (*) and starts to proliferate to form the neuroblast layer of the embryonic neural retina (nr). F. During fetal development the lens and retina continue to grow and differentiate. Lens proliferation becomes restricted to the epithelium (arrow) of the germinative zone (gz) above the equator (thick dashed line) and fiber differentiation is initiated in the transitional zone (tz) below the equator. In the neural retina, the ganglion cells (gc) are the first to differentiate from the neuroblast layer (nb). Abbreviations: aq, aqueous chamber, c, cornea; cb, ciliary body; on, optic nerve; i, iris; vit, vitreous chamber. Thin dashed lines demarcate the approximate boundaries of the germinative zone and transitional zone, either side of the equator. Scale bar: (A, B) 63 μm; (C, D) 100 μm; (D, E) 156 μm; (F) 212 μm.

gamma-crystallins, MIP, filensin), whereas the anterior lens vesicle cells that face the cornea differentiate into a cuboidal epithelial monolayer, the lens epithelium (91). The continued elongation and differentiation of the primary fiber cells reduces the size of the lumen of the vesicle until the apical surfaces of the fibers contact the apical surfaces of the anterior epithelium (figure 2). From this stage the lens has assumed its distinctive polarity and the two populations of cells, the fibers and epithelium, will maintain this spatial relationship throughout life. As the lens grows and matures, the epithelial cells retain the ability to proliferate in a restricted region just anterior to the lens equator, the germinative zone of the lens; the more central epithelial cells becoming increasingly quiescent. Progeny of cell divisions in the germinative zone, move either anteriorly where they contribute to the epithelium or posteriorly across the equator into the transitional zone, where they elongate and differentiate into secondary lens fiber cells, which continue to be added in layers around the central primary fibers throughout life (figure 2). Thus the youngest fibers of the lens are in the outer cortical layers, whereas the oldest fibers are in the central core or nucleus of the lens. Terminal differentiation of these central fibers is characterized by the appearance of cell surface specializations, the expression of fiber specific proteins and the loss of cell nuclei and organelles (86, 87, 113).

Several growth factor families have been implicated in regulating lens differentiation (86, 87). These include FGFs, BMPs, TGFbetas, retinoic acid, insulin/IGFs, platelet derived growth factor (PDGF) and tumor necrosis factor alpha (TNFalpha) at various stages of lens development (86). While numerous factors have been shown to stimulate lens epithelial cell proliferation, a key

finding has been that a gradient of FGF stimulation in the eye can account for the patterns of proliferation, migration and fiber differentiation that occur in the lens (86, 87, 113). To date, FGFs are the only known molecules that can induce lens epithelial cells to adopt a fiber cell fate. While FGFs have been shown to be essential for initiation of fiber differentiation (114) they are not the only players involved. Members of the TGFbeta superfamily have been shown to be important in regulating different phases of fiber differentiation; BMPs have been shown to be required for fiber cell elongation (115, 116), whereas TGFbeta signaling is essential for terminal differentiation (117). More recently, increasing evidence has accumulated for Wnt/Fz signaling playing important roles in eye field specification, lens differentiation and also retinal cell biology (see below).

8.2. Retinal differentiation

Differentiation within the optic cup involves a complex interplay between intrinsic and extrinsic cues that regulate neuroblast proliferation and differentiation of the seven cell types in the laminated retina (118-120). Within the retina, neurogenesis proceeds from the centre of the retina, near the optic nerve, to the peripheral parts of the optic cup. These central to peripheral waves of proliferation and differentiation within the neuroblast layer generate specific neurons in a defined chronological sequence; retinal ganglion cells differentiate first, followed by horizontal, cone photoreceptor, amacrine, rod photoreceptor, bipolar and Muller cells in overlapping phases. Within the neuroblast layer, cytokinetic movements of progenitor cell nuclei correlate with stages of the cell cycle; S-phase occurring in the ventricular zone and M-phase at the ventricular surface. The retinal progenitors retain multipotency during neurogenesis, with daughter cells often adopting different fates. In fish and amphibians, the retina continues to grow by the addition of retinal cells that are generated from undifferentiated progenitor cells peripherally in a region known as the ciliary marginal zone (CMZ). Recent studies have suggested the existence of similar populations of progenitor stem cells in the pigmented ciliary margin (PCM) of birds and possibly mammals (121, 122).

Various secreted factors and cell-associated signaling (Shh, FGF, BMP, TGFalpha, Notch), as well as several classes of transcription factors (Pax6, Otx2, Vax, Pax2, Rx, Mitf, Chx10), have been shown to be important in specifying retinal cell types. A key family of secreted molecules in this regard is the Hedgehog family. Shh, which can regulate the expression of several key retinal transcription factors (Pax6, Vax, Rx, Mitf), plays multiple roles during eye morphogenesis (120). Gradients of Shh signaling within the neuroepithelium are thought to regulate formation of various ocular structures (optic cup, optic stalk, RPE) along the proximo-distal and dorso-ventral axes, reminiscent of the partitioning by Shh of the *Drosophila* eye imaginal disc. Similarly, progression of the central-peripheral wave of neurogenesis in the vertebrate zebrafish retina, like the progression of the morphogenetic furrow in *Drosophila,* seems to be dependent upon hedgehog activity. Moreover, expression of Shh by differentiated ganglion cells acts as a negative feedback signal to modulate the subsequent production of other ganglion cells from the progenitor pool. Superimposed on the Shh gradients are opposing gradients of BMP signals, which serve to antagonize Shh signals, particularly in specifying the D-V axis in the developing retina and also the projection of ganglion cell axons (120, 123). BMP and TGFbeta signals have also been implicated in retinal cell survival/apoptosis and neuronal differentiation at different stage of retinal development (120).

In fish and amphibians, significant retinal neurogenesis during embryonic development and in adult life also occurs at the anterior retinal margin, in the CMZ (122). Recently a similar, albeit smaller, region has been identified in the peripheral retina of postnatal chicks (124), where cells incorporate bromo-2'-deoxyuridine (BrdU) and express transcription factors (*Pax6*, *Chx10*) characteristic of retinal progenitors. Analogous regions have thus far not been identified in the mouse and it has been proposed that the CMZ has been gradually lost during evolution (121). However, dissociation cultures of the pigmented ciliary body from rats and mice have revealed the presence of multipotent retinal progenitor cells (122, 125, 126), raising the possibility that analogous neurogenic stem cells exist in the retina of adult mammals. Given the potential for utilizing such 'stem' cells in treating human disease, there is significant interest in the molecular mechanisms that regulate stem cells in the CMZ of lower vertebrates and neural stem cells in mammals. Similar to its role in regulating retinal progenitors in the central retina, Shh also regulates the proliferation of PCM progenitors in the chick (127). Interestingly, in mice with a heterozygous mutation of the Patched receptor ($Ptc^{+/-}$) there is a zone of mitotically active cells in the marginal retina that express progenitor markers and resembles the CMZ (128). Recent studies indicate that, in addition to Shh signals, Wnt/Fzd signaling also plays important roles in regulating retinal progenitors in the CMZ of the chick (see below).

9. WNT/FZD SIGNALING AND VERTEBRATE EYE DEVELOPMENT

9.1. Wnt/Fzd signaling during eye specification

Evidence for the involvement of Wnt/Fzd signaling in the early stages of eye specification has until recently appeared contradictory. Most of the studies of eye specification have been carried out in *Xenopus* and zebrafish embryos as gastrulation and formation of the ANP can be easily studied and manipulated by injection of capped mRNAs and morpholinos at the blastula stage. Moreover, the availability of mutants (naturally occurring or ENU-induced) with eye phenotypes has permitted the identification of genes important in eye development. Studies of zebrafish mutants (*masterblind* and *headless*) with mutations of *axin* and *Tcf3* have indicated that canonical Wnt signaling inhibits eye formation (129, 130). In the *headless* mutant, a mutation of *Tcf3*, which is a transcriptional repressor of Wnt target genes (131), causes loss of eyes, forebrain and part of the midbrain (130). In the *masterblind* mutant, a mutation in *axin* results in re-specification of the anterior eye and telencephalon

structures into more posterior diencephalon (129). Similarly, injection of agonists of the Wnt pathway, such as a dominant-negative form of GSK-3beta or a form of axin that is able to bind and inhibit GSK-3beta, results in eye reduction or loss (132). Nambiar and Henion (132) have recently identified an ENU-induced zebrafish mutant, *colgate*, with posteriorization of the ANP and reduced eyes, which could be rescued by injection of Wnt antagonists such as GSK-3beta and axin, suggesting that the *colgate* mutant activates the Wnt pathway. The identity of the *colgate* gene is still not known. Conversely, injection of various antagonists of the canonical Wnt pathway, such as Dkk1 (132, 133), into wild-type early zebrafish embryos result in larger eyes, suggesting that canonical Wnt signaling inhibits eye formation. Consistent with this, Kim and colleagues (134) showed that over-expression of *Wnt8b* or treatment with LiCl, which mimics canonical Wnt signaling, resulted in loss of anterior structures (including eyes) and the loss of *six3* and *rx1* expression. Thus, similar to *Drosophila*, Wnt signals in vertebrates appear to be important in delineating boundaries between anterior head structures and the eye field.

During mid-late gastrulation various *Wnt* (*Wnt1, Wnt4, Wnt 8b, Wnt10b* and *Wnt11*) and *Fzd* (*Fzd3, Fzd5, Fzd8a*) genes are expressed in the zebrafish ANP. *Wnt1, Wnt8b* and *Wnt10b* are expressed in domains caudal to the eye field (delineated by *Rx3*), whereas *Wnt11* expression in a broad domain, overlaps part of the eye field (see (135) and references therein). *Fzd8a* expression domain overlaps the expression of several anterior neural ectoderm markers including *Six3*, which is expressed in the presumptive eye fields (134, 136). *Fzd5* expression domain appears to completely overlap the eye field delineated by *Rx3* (135). *Fzd3* has been documented in Xenopus ANP in a domain that overlaps with neural domains of the presumptive eye (137, 138).

Surprisingly, overexpression of *Fzd3* receptors in pre-gastrulation Xenopus embryo, which would be expected to activate Wnt/Fzd signaling, results in ectopic expression of eye homeodomain transcription factors *Pax6, Rx*, and *Otx2* and in ectopic eye formation (138). Conversely, over-expression of an inhibitory form of Fzd3 (extracellular CRD domain) or an intracellular binding protein (Kermit) disrupted the expression of these homeodomain proteins and inhibited normal eye formation. These results initially appear to be at odds with the data indicating that Wnt signaling inhibits eye formation. However, it has been suggested that the action of Fzd3 is predominantly through non-canonical Wnt signaling as over-expression of *Fzd3* did not lead to effects on axis duplication, which is initiated by canonical Wnt signaling. A recent series of elegant experiments by Cavodeassi and colleagues (135) show that specification of the eye fields in the ANP arises through mutual antagonism of the canonical and non-canonical pathways. Using transplants of cells, which over-expressed *Wnt8b* and *Wnt11*, into the developing eye field of gastrula stage embryos they showed that Wnt11 promoted but Wnt8b and other activators of Wnt/beta-catenin signaling inhibited eye formation and the expression of key eye field markers (*Rx, Otx2, Six3*). While the specificity of Wnt/Fzd interactions is still unclear, they showed by genetic interactions that *Fzd8a* facilitates the *Wnt8b* effects and *Fzd5* facilitates *Wnt11* signaling. Moreover they showed, using morpholino approaches, that the two pathways antagonize each other such that over-activation of either pathway has effects on the eye field and activation or inhibition of both negates each other's effects. They propose that non-canonical signaling via Wnt11/Fzd5 activity plays a key role in specifying the eye field and defining the posterior boundary of the eye field by suppressing posteriorizing Wnt/beta-catenin signals. Wnt11/Fzd5 activity may also initiate early morphogenetic events by regulating planar cell movements and maintaining the coherence of the eye field. Consistent with this, *Fzd5* is expressed in the developing retinal eye field at the neural plate stage in both *Xenopus* (139) and zebrafish (135). Another candidate non-canonical Wnt that may regulate eye formation is *Wnt4*. *Wnt4* is expressed adjacent to the eye field and morpholino inhibition of its expression results in loss of eyes, which can be rescued by expression of a disheveled mutant lacking the DIX domain but not by expression of beta-catenin (140). One of the targets of Wnt4 appears to be the RNA polymerase II elongation factor, EAF2, which also is required for eye development and regulates expression of Rx. Studies in the Medaka fish indicate that *Sfrp1*, which is expressed in the ANP and the developing optic vesicles, may also activate the non-canonical Wnt signaling during eye-field specification as down regulation of *Sfrp1* decreases, whereas over-expression of *Sfrp1* increases the eye field (141).

Further evidence for the inhibitory effect of Wnt/beta-catenin signaling on eye specification and lens induction in ectoderm comes from studies of mice in which beta-catenin was conditionally mutated in *Pax6*-expressing periocular ectoderm. Cre-LoxP-mediated deletion of beta-catenin, which would be expected to inhibit Wnt/beta-catenin signaling, resulted in the formation of ectopic lenses, whereas an activating mutation of beta-catenin suppressed lens formation in presumptive lens ectoderm (142). Similar to *Drosophila,* BMPs are also essential for vertebrate eye formation (143-145). However, while *Dpp* is known to suppress *Wg* in the *Drosophila* eye field (50), and BMP7 regulates *Wnt4* expression in the kidney (143), it is not known how the BMPs involved in eye induction (BMP4 and BMP7) affect Wnt expression or signaling in the vertebrate eye field.

The importance of inhibiting Wnt signals for specifying the eye field and retinal cells has recently been highlighted by *in vitro* experiments, which have shown that the specification of neural retinal precursors from embryonic stem cells can be recapitulated by using a combination of Wnt (Dkk1) and Nodal (LeftyA) antagonists followed by serum and activin treatment (146). These precursor cells expressed characteristic eye markers (*Rx* and *Pax6*) and could be induced to form photoreceptors in reaggregation cultures with embryonic retinal neurons and were able to survive and integrate in organotypic cultures to form photoreceptors. It remains to be seen whether such precursor cells can be applied to *in vivo*

models in transplantation experiments, particularly to models of retinal photoreceptor degeneration.

9.2. Wnt/Fzd expression during vertebrate lens development

Recent expression studies have documented various components of the Wnt signaling pathway during lens morphogenesis and differentiation. One of the earliest studies documented the expression of chicken *Wnt13* (now known as *Wnt2b*) in the developing chick eye, coinciding with proliferating epithelial cells in the developing lens and a dynamic pattern in the developing optic cup and peripheral retina (147). During early lens morphogenesis, *Wnt2b* expression was found in the presumptive lens ectoderm that will form the lens placode and in the dorsal optic vesicle that is in close proximity to the overlying ectoderm. At the lens vesicle stage, expression is restricted to the lens epithelium and dorsal pigmented epithelium of the retina. As ocular morphogenesis proceeds, it is retained in the lens epithelium but is gradually lost from the developing ciliary body and iris. Since this study, expression of various *Wnt, Fzd, Dkk* and *Lef/Tcf* genes has been identified in the developing eye of various species by RT-PCR, *in situ* hybridization and immunohistochemistry (139, 147-157).

In situ hybridization studies of several Wnt (*Wnt5a, Wnt5b, Wnt7a, Wnt7b, Wnt 8a, Wnt8b*) genes during murine lens morphogenesis showed that expression patterns for all these Wnts were very similar, with only *Wnt5a* showing a slightly different expression pattern in the mature lens (148, 150) at postnatal (P) day 21. The Wnts appear to be weakly but ubiquitously expressed in the lens and retinal primordia and also periocular mesenchyme at the lens placode (E9.5), lens pit (E10.5) and early lens vesicle (E12.5) stages. As the lens develops and differentiates further through the late lens vesicle stage (E14.5) to fetal (E18.5) and postnatal (P2, P21) stages, the expression patterns become more distinct. Expression of these Wnts (and also *Dkk* and *Fzd,* see below) becomes restricted to the lens epithelium and is down-regulated as cells exit the cell cycle and initiate differentiation into lens fiber cells. Exceptions to this pattern are *Wnt7b*, which continues to be expressed in the cortical fibers of the lens undergoing terminal differentiation, and *Wnt5a*, which is not expressed in the mature anterior lens epithelial cells and is restricted to cells in the equatorial region where cells are proliferating and undergoing early stages of differentiation. Similar expression studies for *Wnts 1, 2, 3, 4, 5a, 5b, 7a, 7b* and *Wnt13* were conducted by Liu and colleagues (152) at early (E12.5) and late (E14.5) lens vesicle stages of eye development. They demonstrated transient expression of *Wnt3* in the lens epithelium at E12.5 and little or no expression of *Wnt1, 2* or *4* at either stage. However, their findings for *Wnt5a, 7a* and *7b* differed somewhat from those described above. While they showed quite similar expression patterns for Wnt5b to the McAvoy group (148, 150) their data showed little or no expression of *Wnt5a* at these stages of lens development. Moreover, *Wnt7a* was only detectable after closure of the lens vesicle at E14.5 and was expressed almost exclusively in the fiber cells. *Wnt7b*, on the other hand, was not detected in the lens and only detected transiently and weakly in the optic cup. As the probes used in the various studies appear to be specific it is hard to explain these differences. It is possible that variations in tissue preparation between the two studies may have led to these different results. Further studies will be needed to clarify these discrepancies. Most recently, Fokina and Frolova (157) have completed a comprehensive study of the Wnt genes during chick eye development. They show that *Wnt2* and *Wnt2b* are expressed in the chick lens epithelium, while *Wnt5a, Wnt5b, Wnt7a, Wnt7b* are expressed in the differentiating lens fibers and suggest that there may be species differences as to which Wnts are expressed. While the precise distribution of transcripts for some of the Wnts is still unclear, the data from these studies show that multiple Wnts are expressed in the lens and suggest they play roles during morphogenesis and differentiation.

Consistent with the presence of Wnts, the expression of Fzd receptors (*Fzd1-8*), co-receptors (*Lrp5, Lrp6*), *Sfrp1-3* and *Dkk1-3* genes have also been identified during rodent lens morphogenesis (148-150, 152, 158). Essentially similar expression patterns have been documented for most of the Fzd genes (*Fzd1, 2, 3, 4, 5, 7, 8*) in the lens, with expression being predominantly restricted to the epithelial layer from E12.5- E18.5 (149, 152). By contrast, *Fzd6* was not detectable in the epithelium and became increasingly expressed in differentiating fibers (152).

All five of the known *Sfrp* genes, have been detected during lens development by various investigators (149, 152, 159, 160). While originally identified as being able to bind Wnts and antagonize signaling, Sfrps also have the capacity to potentiate Wnt activity (7) and can also bind to Fzd receptors. Interestingly, the Sfrps show very different patterns of expression in the developing lens. *Sfrp1* expression appears as the lens starts to differentiate at the lens vesicle stage and is subsequently detected in both epithelial and fiber cells of the lens (149, 152, 159). *Sfrp2* is first detected as the lens ectoderm forms the placode and is a key marker for this stage of development (145). During subsequent lens morphogenesis *Sfrp2* expression is restricted to the lens epithelial cells (149, 152, 159). There is some discrepancy between several studies as to whether *Sfrp3* is expressed during lens development. Liu *et al.,* (152) reported not being able to detect *Sfrp3* by *in situ* hybridization. However, Chen and colleagues (149) report that *Sfrp3*, was detected from E14.5 in a pattern similar to that seen for *Sfrp1* and also *Sfrp4* (149, 159). Three of the four known *Dkk* genes *(Dkk1-3)*, which also can act either as agonists or antagonists of Wnt signals (7), have been identified in the developing lens with expression patterns similar to that described for many of the Wnts and Fzd genes (148).

In summary, most of the Wnt signaling pathway genes are expressed predominantly in the developing lens epithelium with several genes (*Wnt5a, Wnt7a, Fzd3, Fzd6* and *Sfrp1*) showing marked expression in differentiating fiber cells.

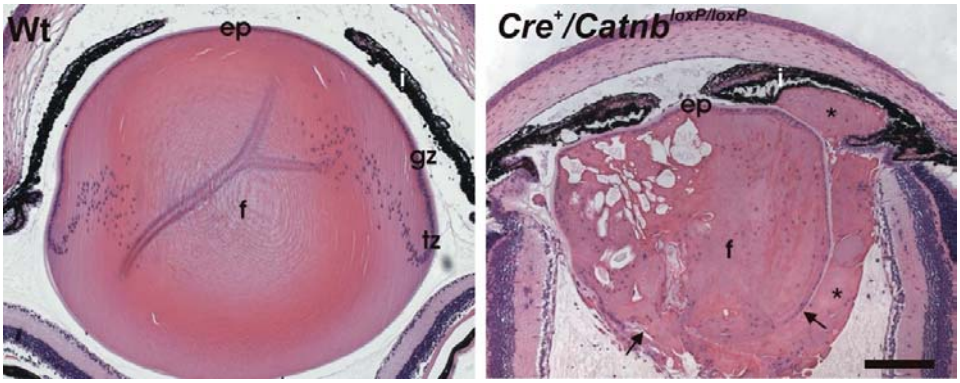

Figure 3. Loss of beta-catenin affects lens differentiation.Sections show wild type (Wt) and mutant ($Cre^{+}/Catnb^{loxP/loxP}$) lenses at postnatal day 21. Wild type lens has a normal epithelium (ep), germinative zone (gz), transitional zone (tz) and fiber mass (f). Disruption of beta-catenin in the mutant lens results in disruption of the lens structure, particularly the gz, tz and the fiber mass. The basement membrane of the lens, the lens capsule, is ruptured posteriorly (arrows), resulting in extrusion of fiber cells (*) into the ocular media and under the iris (i). Scale bar, 100 μm.

9.3. Role of Wnt/Fzd signaling in lens development

The expression of *Wnt2b* and *Sfrp2* in the lens placode and the effects of deleting beta-catenin in the ocular ectoderm (142) indicate that Wnt signals play an important role during lens induction and early morphogenesis. However, the precise roles of Wnt/Fzd signals and where they sit in the gene hierarchy that regulates these processes are not yet clear. Analyses of $Pax6^{-/-}$ and $Bmp7^{-/-}$ mice show that *Sfrp2* expression is regulated by *Pax6* and that it may play a role in the interactions between BMP signaling and Pax6 expression during lens placode formation (145). Recent data places the *Homothorax* orthologs, *Meis1* and *Meis2*, as upstream positive regulators of *Pax6* during vertebrate lens placode formation (161). In *Drosophila*, *Homothorax* functions as a negative regulator to delineate the eye field (162) and in combination with *Ey* and *Teashirt* (*Tsh*) regulates eye disc cell proliferation (163). While *Homothorax* is regulated by *Wg* in *Drosophila,* it is not known whether its vertebrate orthologs, *Meis1* and *Meis2*, are regulated by Wnts.

The predominantly epithelial expression of *Wnt* and *Fzd* genes during subsequent lens morphogenesis and differentiation is consistent with studies of a Wnt reporter transgenic mouse (152). The transgene contains six LEF/TCF response elements that drive *LacZ* expression and thus permits identification of tissues where the Wnt signaling pathway and TCF-mediated transcription are active. Analysis of these *Tcf/Lef-LacZ* mice shows that there is transient activation of Wnt signaling in the anterior lens epithelium between E13.5 and E14.5 after closure of the lens vesicle (152). Consistent with this, studies of mice with a null mutation for *Lrp6*, which would be expected to inhibit the canonical Wnt pathway, reveal a marked deficit of the anterior lens epithelium, resulting in rupture of the lens fibers into the overlying cornea (150). By contrast, treatment of sub-confluent lens epithelial explants with LiCl, which mimics Wnt/beta-catenin signals, promoted apical basolateral polarity and essentially maintained the normal lens epithelial phenotype (164). These data indicate that the canonical Wnt pathway plays key roles in regulating the formation and maintenance of the lens epithelium. However, several lines of evidence indicate that Wnt signals also play key roles in the differentiation of the lens fibers. The active (non-phosphorylated) form of beta-catenin and inactivated GSK3beta can be found in lens fiber as well as epithelial cells (150, 153, 165). *In vitro* analyses indicate that Wnt3a-conditioned medium and LiCl can induce the accumulation fiber-specific beta-crystallin, in epithelial cells but do not induce the characteristic cell elongation (153). By contrast, when epithelial cells are pre-treated or primed with low levels of FGF, Wnt3a-conditioned medium, but not LiCl, can induce the fiber elongation response (153), suggesting that non-canonical Wnt signals may also be involved. Recent studies in our laboratory (165), of mice with conditional deletion of beta-catenin in different lens compartments, show that beta-catenin is not only required to maintain the integrity of the lens epithelium but also for differentiation of epithelial into fiber cells. Lenses lacking beta-catenin show disrupted fiber differentiation and the posterior capsule is ruptured (figure 3). A similar phenotype is seen in transgenic mice that over-express *Sfrp2* in the lens fibers (166).

Overall, these results suggest that both canonical and non-canonical Wnt signals are operating during fiber differentiation: beta-catenin-dependent canonical signals appear to regulate crystallin accumulation, whereas non-canonical signaling may be involved in the cytoskeletal rearrangements involved in fiber cell elongation.

Wnt/Fzd signaling has also been shown to play a role in the regression of the ocular fetal vasculature, particularly the hyaloid vasculature and the PM/TVL of the lens. Mice with a loss of function mutation of *Lrp5*, *Fzd4*, or *Lef1* display persistence of the fetal (hyaloid and PM/TVL) vasculature postnatally (167-169). Similarly, mice with hypomorphic Wnt7b alleles display persistent ocular vasculature (168). It has been proposed that macrophage-derived Wnt7b activates canonical Wnt signaling in the vascular endothelial cells, via Fz4 and Lrp5, and couples cell cycle entry to apoptosis and thus mediates regression of the fetal vasculature (168).

9.4. Wnts/Fzd expression during retinal development

The most comprehensive analysis of Wnt and Fzd expression in the developing mouse retina has been carried out by Liu and colleagues (152). RT-PCR identified Wnt*1, -3, -5a, -5b, -7b* and *-13* (*Wnt2b*) in embryonic, and fetal retinae and *Wnt5a, -5b, -10a* and *–13* in the adult retina. *In situ* hybridization of eye sections from E12.5 and E14.5 mice showed a dynamic central-peripheral expression of *Wnt3, -5a, -5b, -7b* in the neuroblast layer. Initially, at E12.5, the Wnts were diffusely expressed throughout the neural retina but by E14.5 were down regulated in the central retina but still detectable in the peripheral retina. The expression pattern for *Wnt13* (*Wnt2b*) was similar to that documented in the chick (147, 170), with expression predominantly detected in the RPE. By contrast, Jin *et al.,* (156) documented the expression of *Wnt 3, Wnt5a* and *Wnt11* predominantly in the RPE of the Stage 25-27 chicken retina. At these stages, the chick retina consists of a relatively undifferentiated neuroblast layer and a retinal pigmented epithelial monolayer similar to that seen in fetal mouse retina. In the differentiating postnatal (P7) mouse retina, *Wnt5a, Wnt5b* and *Wnt13* were expressed in the inner nuclear layer in amacrine cells, identified by co-staining for Pax6. *Wnt13* was also detected in the ganglion cells along the inner retina. Similar expression patterns for *Wnt5b* and *Wnt13* were detected in the adult retina; however *Wnt5a* was not detected (152).

In the embryonic mouse retina at E12.5 expression of *Fzd3, Fzd4, Fzd6* and *Fzd7* were detected throughout the optic cup and *Fzd4* was detected in the RPE (152). However, the patterns changed markedly during subsequent morphogenesis, particularly in the peripheral margin of the optic cup that is destined to form the ciliary body and iris. At the lens vesicle stage (E14.5), *Fzd4, Fzd6* and *Fzd7* expression became restricted to this ciliary marginal zone, whereas Fzd3 expression becomes down-regulated in the ciliary marginal zone while being maintained in the neural retina. During subsequent differentiation of the retina at P7, *Fzd3, Fzd6* and *Fzd7* are expressed in the ganglion cells and inner nuclear layer, whereas Fzd4 is expressed in all three cellular layers. In the adult retina, Fzd expression becomes increasingly diffuse with *Fzd3*, *Fzd6* and *Fzd7* more restricted to the inner nuclear layer, whereas *Fzd4* was detected in the inner nuclear layer and the photoreceptors (152). In the chick, similar expression patterns for *Fzd4, Fzd6* and *Fzd7* to the mouse were documented during early (optic cup) and later, differentiating stages of the retina (155). Expression patterns for *Fzd1, Fzd2, Fzd5*, *Fzd8* and *Fzd9* were also documented; at the optic cup stage, *Fzd1* expression, like *Fzd4, Fzd6* and *Fzd7*, became restricted to the ciliary marginal zone, whereas *Fzd2, Fzd5* and *Fzd8* were predominantly expressed in the neuroblast layer of the neural retina. In the mature retina (St 42), *Fzd5* was not detected and *Fzd1, Fzd2, Fzd3, Fzd6, Fzd8* and *Fzd9* were all detected in the inner nuclear and ganglion cell layers and *Fzd1, Fzd2, Fzd6, Fzd8* and *Fzd9* were present in the ONL/photoreceptor layer (155). In Xenopus, *Fzd5* is expressed during early ocular morphogenesis in the retinal disc of the optic vesicle and during subsequent differentiation of the optic cup into the laminated retina becomes restricted to the peripheral ciliary margin (139, 171).

Studies of *Sfrp* gene expression (152, 156, 159) show that these genes have very distinct patterns of expression that are similar in mice and chicks. During early morphogenesis, *Sfrp1* is expressed predominantly in the RPE. By contrast *Sfrp2* and *Sfrp4* are expressed in the neural retina but markedly downregulated in the ciliary margin. *Sfrp3* is not detected until just before birth. In the differentiating retina *Sfrp1* expression is detected in all cellular layers but *Sfrp2* and *Sfrp3* show complementary patterns in the inner nuclear layer, with *Sfrp2* expressed in the more immature peripheral retina and *Sfrp3* in the more mature central retina.

9.5. Role of Wnt/Fzd signaling in retinal development

The dynamic expression pattern of *Wnt*, *Fzd* and *Sfrp* genes in the developing retina suggest that Wnt/Fzd signaling has several different roles that may involve canonical and/or non-canonical pathways. Evidence for involvement of the Wnt/beta-catenin pathway comes from analyses of the *Lef/Tcf-LacZ* reporter mice, which showed there was transient activity in the pigmented ciliary margin, RPE and neuroblast layer, particularly in the peripheral optic cup at E13.5 and E14.5. (152). Similar reporter analyses show extensive activation of Wnt signaling in the CMZ of zebrafish (172) and *Xenopus* (171). In the mouse, the regions of strongest LEF/TCF activity coincided with the expression of *Wnt13* (*Wnt2b*), *Fzd4* and *Lef1* in the CMZ (152, 170). Over-expression of murine *Wnt2b*, by *in ovo* electroporation, in the optic cup of the early chick eye resulted in expansion of the neural progenitor pool but inhibition of neurogenic markers and neural differentiation in the central retina due to ectopic activation of Wnt/beta-catenin signaling (170, 173). Similar findings have been found in zebrafish retina (172). By contrast, electroporation of a dominant-negative form of *Lef1*, designed to inhibit Wnt/beta-catenin signaling, resulted in inhibition of proliferation and precocious neural differentiation. Further studies, using a combination of *in ovo* retroviral infection of optic vesicle cells and *in vitro* explant approaches (173), show that Wnt2b maintains the proliferation of retinal progenitor but inhibits the expression of neurogenic genes (*Cath5*, *NeuroM*, *Cash1*, *Ngn1* and *Ngn2*) and also *Notch1*, independently of Notch activity. These results suggest that Wnt/beta-catenin signaling regulates proliferation of CMZ progenitor cells, preventing them from undergoing differentiation to form the neural retina or non-neural anterior eye structures (e.g. iris and ciliary epithelium). In the currently proposed model, Wnt 2b maintains cells in the CMZ as undifferentiated retinal progenitors whereas cells in the central retina or those that leave the CMZ no longer receive the Wnt signal and initiate expression of proneural genes and *Notch1*, which trigger neural differentiation.

However, recent studies on the role of signaling via the *Fzd5* receptor in *Xenopus* retina (171) indicate that canonical Wnt signals not only result in proliferation of retinal progenitors but also initiate differentiation of neuroblasts. Morpholino knockdowns show that expression of *Sox2*, a transcription factor involved in eye-field

specification and neurogenesis, is dependent upon *Fzd5*. Inhibition of *Fzd5* or *Sox2* down-regulated several proneural genes and caused retinal progenitors to adopt a glial (Muller cell) fate. Van Raay (171) proposed that the commitment of progenitor cells in the ciliary marginal zone to a purely neural retina fate is associated with the expression of *Fzd5* and *Sox2*. The slightly different results documented in *Xenopus* and chick may reflect different roles for the CMZ in these species and also that *Fzd5* appears to have different expression patterns in chick (155) and *Xenopus* (139, 171).

In *Drosophila*, *wg* induces *Iroquois* expression in the dorsal eye compartment and combines with *Iro-C* to promote dorsal rim ommatidia formation. While Wnt signals appear to regulate *Iroquois* gene expression (174) in the *Xenopus* ANP, there as yet appears to be no evidence for *Wnt-Iroquois* interactions in mammalian eye field specification or in retinal differentiation. The six vertebrate *Iroquois (Irx)* genes are expressed in post-mitotic neurons (eg. ganglion cells, bipolar cells) of the developing postnatal retina (175-178) but interactions with the Wnt/Fzd signaling pathway have not yet been demonstrated.

Recent data link Wnt and hedgehog signaling via PKA activation in the regulation of neurogenesis and differentiation in zebrafish retina (172). Similar to chick and frog, the Wnt/beta-catenin signaling pathway is essential to mediate cell proliferation in the zebrafish retina, particularly in the CMZ. Activation of protein kinase-A (PKA) appears to prevent progenitors from exiting the cell cycle during Wnt-activated proliferation and also acts as a negative regulator of hedgehog signals, which are involved in cell cycle exit by regulating the expression of the cyclin-dependent kinase inhibitor, p27, and neural differentiation through expression of *atonal* (*Ath5*). Thus PKA is proposed to act as a switch between proliferation and differentiation.

Further *in vitro* studies suggest that Wnt signaling via *Wnt2b* is also involved in retinal lamination. In re-aggregation cultures, dissociated retinal cells usually form rosette structures, which lack normal retinal lamination unless glia or RPE cells are present (179, 180, 181). Using quail explants and chick re-aggregation cultures, it was found that explants of ciliary margin could also reorganize rosette-forming cells into a two-dimensional epithelial sheet that exhibited correct retinal lamination (182). Moreover, this effect was mimicked by addition of Wnt2b to dissociated cultures and inhibited by Wnt antagonists (e.g. soluble Fzd5 extracellular domain). It was proposed that the anterior rim of the optic cup functions as a retinal layer-organizing centre via Wnt2b. Evidence for Wnts having such a role *in vivo* comes from experiments in *Xenopus*, which demonstrated that over-expression of Wnt antagonists (*Sfrp2* or *Frzb*) in embryos resulted in disruption of retinal lamination (183).

Vascularization of the retina also appears to involve Wnt/Fzd signaling. Mice with a null mutation for Fzd4 show defective development of the primary network that spreads across the inner retina and the secondary intraretinal capillary networks in the outer and inner plexiform layers (169). Moreover, the primary vessels appeared to have abnormal fenestrations. A similar phenotype is seen in mice with a targeted mutation of the *norrie disease protein* (*Ndp*) gene which encodes the norrin protein (184, 185). It has been proposed that norrin acts as a high affinity ligand for Fzd4 in the retina and that it may activate both canonical and non-canonical pathways to regulate endothelial cell proliferation and migration (169). It is interesting to note that the effects of Wnt/Fz signaling on the retinal vasculature are different from the effects on the hyaloid vasculature and TVL of the lens. Disruption of norrin/Fzd4 signals results in failure of retinal vessel formation but disruption of Wnt7b/Fz4 signals prevents vessel loss in the hyaloid and lens vasculatures (see above). Disruption of these Fzd4 signaling pathway has significant consequences for human eye disease (see below).

10. HUMAN OCULAR DISEASE AND THE WNT SIGNALING PATHWAY

Several human ocular pathologies are caused by mutation of known Wnt pathway genes and genes that may regulate or be affected by this pathway.

Familial exudative vitreoretinopathy (FEVR) is a genetically heterogeneous disorder characterized by the incomplete growth of peripheral retinal capillaries leading to fibrovascular mass lesions (186). Clinical features can be highly variable, even within the same family; severe forms of the disease can result in detachment of the retina and legal blindness, while mild forms are asymptomatic. Families with FEVR display several forms of inheritance. The most common form is autosomal dominant (187, 188), but autosomal recessive (189, 190) and X-linked inheritance have also been described (191-193).

In studies of several large kindreds, three autosomal dominant loci have been identified: *EVR1* (11q13-q23) (188), *EVR3* (11p12-13) (194) and *EVR4* (11q13-q23) (195). There is also evidence that there may be other loci (196). Mapping of mutations at 11q13-q23 (*EVR1*) defined deletions within the Wnt receptor *FZD4* gene (197, 198), which account for approximately 20% of FEVR cases (199). Mutations mapped to *EVR1* were also detected in the closely linked *LRP5* gene which encodes a Wnt co-receptor (194, 198). Analysis of the mutations in 3 families with autosomal recessive FEVR also showed linkage to chromosome 11q (200). Sequencing of the *LRP5* gene within this region identified 3 mutations within this gene (R570Q, R752G and E136K) (200) indicating that mutation of *LRP5* can be responsible for both dominant and recessive forms of FEVR. Recessive loss-of function mutations of LRP5 have been shown to give rise to osteoporosis-pseudoglioma syndrome, which is characterized by failure of regression of the fetal eye vasculature (hyaloid and PM/TVL) (201) similar to that seen in mice with a null mutation of *Lrp5* (167). Mapping of X-linked FEVR kindreds (*EVR2*) identified missense mutations in the Norrie disease gene in one family (191, 202). However, studies in other X-linked FEVR families

have failed to identify mutations in the Norrie disease gene suggesting other loci are also involved (190).

Norrie disease is an X-linked disorder characterized by retinal dysplasia associated with hearing loss and mental retardation that often results in blindness at birth. The gene underlying Norrie disease was isolated by positional cloning (203, 204). Norrin, the protein product of the Norrie disease gene is a secreted protein containing a cysteine knot motif. The similarity of the vascular phenotypes observed in *FZD4* and *Norrin* mouse mutants and human disorders prompted studies on the interaction between Norrin and FZD4 (169, 205). Norrin was found to be a high affinity non-Wnt ligand for FZD4 (169). Binding is very specific as Norrin does not bind to FZDs 3, 5-8. As assessed by super TOP-FLASH assays, the Norrin/FZD4 receptor pair activates the canonical Wnt signaling pathway in the presence of LRP5 or LRP6 (169, 205).

These results implicate the ligand-receptor pair Norrin/FZD4, along with LRP5, in the vascularization of the human retina and contributing to FEVR and Norrie disease when mutated. The genes carrying mutations underlying FEVR in several other kindreds, displaying autosomal dominant and X-linked inheritance, are yet to be identified. It is possible that they may also be components or regulators of the same signaling pathway.

A subtle abnormality of the iris has been observed in patients with Williams-Beuren syndrome, which has been associated with mutations of the *FZD3* gene in humans (206). In one series of 152 patients with Williams-Beuren syndrome, Winter *et al.,* (207) documented strabismus (54%), esotropia (98%) and a stellate iris pattern (74%). While three of these patients (two aged 9 and one 46) also had cataract, this was not characterized further. *FZD3* is broadly expressed in lens, retina, ciliary body and iris during murine development and persists in the postnatal murine retina (152); however, the precise function of signaling via this frizzled receptor in these tissues or how mutations contributes to this disease spectrum is not yet known.

There is a diverse array of inherited retinal degenerative disorders and many different genes and mutations contributing to these diseases have been identified (208). A common feature of retinal dystrophies is the degeneration of retinal photoreceptors by apoptosis. In retinitis pigmentosa, upregulation of secreted frizzled related proteins (SFRP-1, 2, 5) has been observed in association with photoreceptor degeneration and associated apoptosis (209, 210). In these degenerating retinas, the SFRPs were localized to the inner limiting membrane, Bruch's membrane and surviving photoreceptors (210). As SFRPs possess homology to the Wnt-binding cysteine rich domains (CRD) found in frizzled receptors they are thought to primarily antagonize Wnt signaling. However, it is unclear whether the modulation of the Wnt signaling pathway by SFRPs contributes to or is a consequence of these pathologies. Mutational analysis of *SFRP1* in 325 patients failed to identify any mutations associated with retinal dystrophies (211). This suggests that upregulation of SFRP expression rather than alteration of function contributes to retinal degeneration.

One class of human retinal disorders, originally classified as flecked retinal disease (212), is characterized by scattered retinal lesions and includes Stargart's disease, *fundus flavimaculatus* (213) and retinitis *punctata albescens*. These diseases lead to progressive degeneration of the macular area of the retina. Stargart's is the most common form of juvenile macular disease and is likely to occur as a result of homozygous mutation of several loci (214). A similar phenotype, identified by indirect ophthalmoscopy and electroretinography, is found in a spontaneous mouse mutant, retinal degeneration 6 (*rd6)* (215). Homozygous *rd6* mutants exhibit small white lesions across the retina, progressive photoreceptor degeneration and dysfunction of rods and cones (215). Positional cloning of the *rd6* mutation has revealed a splice donor site mutation that results in an in-frame deletion of exon 4 of the membrane-type frizzled related protein (*Mfrp*) gene (216). MFRP is similar to the SFRPs in that it possesses a CRD and is speculated to act as a competitive inhibitor of Wnt signaling (216). However, no direct evidence for this has yet been reported. *Mfrp* is expressed strongly in the pigmented retinal and ciliary epithelia of the eye, which combined with the mutant phenotype in *rd6* mice has made it a candidate gene for human flecked retinal diseases. However, recent studies have demonstrated that null mutation of *MFRP* is responsible for nanophthalmos in humans (217). Nanophthalmos is a recessively inherited disorder, characterized by extreme hyperopia (farsightedness) resulting from insufficient growth along the visual axis, placing the lens too close to the retina. The mechanism by which MFRP affects eye growth and myopia is not clear. It is speculated that the defect in the RPE leads to abnormal development of choroid and sclera from the neural crest or that in postnatal development there is abnormal metabolism of retinoic acid, which has been implicated in hyperopia. The differences in phenotypes observed between mice and humans may reflect the nature of the mutations rather than a difference in function of MFRP in humans and mice, as the mouse mutation is an in frame deletion of 58 amino acids whereas the human mutations appear to be more severe truncations.

Mutations in the *APC* gene are responsible for the autosomal dominantly inherited syndrome, familial adenomatous polyposis coli (FAP) (218), which is characterized by the presence of extensive polyps of the colon and strongly predisposes to colon cancer. The most extensively studied function of APC is its role in the regulation of the WNT signaling pathway (19, 219). Truncation of APC results in dysregulation of beta-catenin, and activation of many different WNT target genes. Gardner syndrome refers to a subgroup of FAP patients with extracolonic manifestations, including congenital hypertrophy of the retinal pigment epithelium (220). These pigmented retinal lesions are often bilateral and multiple (221) and occasionally progress to solid tumors (222). There is some evidence that such retinal lesions are only detected in FAP patients that have truncations in APC after exon 9 (223). These observations suggest that aberrant

activation of the WNT signaling pathway may disrupt retinal architecture.

Other Wnt family members and effectors are also likely to contribute to ocular disease. Homozygous mutation of *WNT3* in humans causes tetra-amelia, which is characterized by a complete absence of limbs and is associated with other defects including cataract and microphthalmia in some cases (224). These ocular defects have not yet been characterized in detail.

11. FUTURE PERSPECTIVES

While a great deal of information is available about the role of Wnt/Fz signaling in *Drosophila* and many lessons have been learned from this model system, the elucidation of the roles these pathways play in the vertebrate eye is still in its infancy. Despite some variations in the genetic pathways that regulate eye formation through evolution, interesting parallels between *Drosophila* and vertebrates are emerging, particularly in eye field specification. Numerous components of the canonical pathway have been identified in the developing eye of several vertebrate species and functional studies have already demonstrated that this pathway plays important roles in lens and retinal differentiation. It is likely that non-canonical Wnt/Fzd signals also play roles in vertebrate ocular development and in this regard studies in *Drosophila* may yet hold important keys for the investigation of these pathways.

The identification of abnormal expression and numerous mutations of genes involved in WNT/FZD signaling attest to the importance of understanding these signaling pathways in the context of human ocular diseases. However, many of the genes that underlie human ocular diseases are yet to be identified and cloned and further studies from both clinical and basic mechanism perspectives are required to elucidate the functions of the numerous WNT/FZD genes that are expressed in the eye. It is likely that investigation of the basic signaling mechanisms of the WNT ligands and FZD receptors in the eye will lead to important insights into their roles in vision and ocular diseases such as retinal degenerations, cataracts, ocular tumors and congenital ocular malformations. The hope is that this knowledge will lead to novel therapeutic paradigms to treat or prevent these debilitating diseases that cause blindness.

12. ACKNOWLEDGEMENTS

The authors acknowledge grant support from NHMRC Australia (GH, HA, RDI), ARC Australia (GH), Retina Australia (HA, RDI) and Melbourne University Research Grants Scheme (RDI). Dr. Jean-Pierre Eid and Sarah Cain are thanked for providing images for figures 1 and 3, respectively.

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Key Words: Cataract, Differentiation, Embryonic development, Eye, Frizzled, Lens, Proliferation, Retina, Retinal degeneration, Retinopathy, Wnt, Wingless, Review

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