Expression of *Drosophila omb*-Related T-Box Genes in the Developing Human and Mouse Neural Retina

Jane C. Sowden,¹ James K. L. Holt,¹ Moritz Meins,¹,2 Hazel K. Smith,³ and Shomi S. Bhattacharya²

PURPOSE. To examine the role of *Drosophila* optomotor blind (*omb*)–related T-box genes in development of human and mouse retina.

METHODS. Mouse *Tbx2*, *Tbx3*, and *Tbx5* cDNAs were isolated from retinal cDNA libraries by hybridization to the *Drosophila omb* gene. Gene expression patterns in developing retina were analyzed by in situ hybridization.

RESULTS. *Tbx2/Tbx2*, *Tbx3/Tbx3*, and *Tbx5/Tbx5* were expressed asymmetrically across the embryonic neural retina with highest levels of mRNA within dorsal and peripheral retina. The dorsoventral gradient of *Tbx2* expression disappeared before the ganglion cell layer (GCL) formed. Its expression then became restricted to the inner neuroblastic retina and later to the GCL and inner nuclear layer (INL). The dorsal expression domains of *Tbx5/Tbx5* and *Tbx3/Tbx3* were maintained during formation of the GCL. As the retina matured, *Tbx3/Tbx3* expression was restricted to the INL, and *Tbx5/Tbx5* was expressed within the GCL.

CONCLUSIONS. The expression pattern of *Tbx2*, *Tbx3*, and *Tbx5* within the developing retina supports the idea that the encoded transcription factors play a role in providing positional information important for topographic mapping and in differentiation of distinct cell types across the laminar axis of the retina. (Invest Ophthalmol Vis Sci. 2001;42:3095–3102)

The mature visual system comprises a complex network of neuronal connections whose physiology and axonal projections are well characterized. In the human retina, more than 100 million rod and cone photoreceptors transmit visual information to some 1.2 million ganglion cells through the bipolar, horizontal, and amacrine interneurons of the inner nuclear layer (INL).¹ The ganglion cell axons project with precise topographic mapping to synaptic targets within the higher visual system. Recent progress has been made in identifying the guidance molecules that ensure the ganglion cell axons navigate to appropriate synaptic targets during development.²–⁴ Less is known, however, about the genetic mechanisms by which ganglion cells and the retinal interneurons achieve their differentiated identities and make precise synaptic connections during development.

Although the visual systems of the cat and the chick have been extensively characterized, these systems are not amenable to genetic analysis. In contrast, the knowledge of the genetic control of the visual system of the fruit fly, *Drosophila melanogaster*, is relatively advanced because of the ease of generating mutant flies.⁵ An emerging strategy that is useful for the identification of genes regulating development of the mammalian visual system is to analyze homologues of *Drosophila* genes that are important in visual system development in the fly.⁹ This strategy is based on accumulating evidence that demonstrates the conservation of genetic mechanisms underlying homologous structures in diverse species and the discovery that mutations in conserved genes underlie inherited eye malformations in flies and mammals.⁷ For example, there is evolutionary conservation of the transcription factors, *Pax6* and *Sine oculis*, which are important in eye development, and of opsins proteins involved in phototransduction.⁹

The pathophysiology resulting from mutation of the *Drosophila* gene optomotor blind (*omb*) demonstrates that the *omb* gene is an important regulator of neuronal cells, which process and integrate visual information from the compound eye and transmit it to the central brain.⁹ In the third larval instar of the fruit fly, *omb* is expressed in the neuroblasts of the optic lobe, which differentiates, which ganglionic neurons with the arrival of axons projecting from the photoreceptor cells.¹⁰ Several *omb* alleles are pupal lethal, and optic lobe neurons fail to differentiate. Other alleles give rise to viable flies with reduced levels of omb protein, resulting in specific behavioral and neuroanatomic defects in the visual system.⁹ These mutant flies do not have large fibers in the lobula plate ganglionic complex of their visual system.

*omb* is of particular interest, because it is a member of an important family of developmental regulators encoded by T-box genes.¹¹,¹² These genes encode transcription factors that share a highly conserved and novel type of DNA-binding domain, the T-box domain. T-box genes have been characterized in a range of species, including humans, indicating the evolutionary conservation of this gene family. Phylegenetic analysis predicts that most animal species have at least five T-box genes.¹² Mutations in several T-box genes cause a variety of developmental defects in mice and humans, thus highlighting the vital role that T-box genes play in diverse developmental processes, including limb and heart morphogenesis and mesoderm formation.¹³–¹⁶

We have focused on the analysis of *omb*-related human and mouse genes to assess whether these genes play significant roles in development of the mammalian retina. Human and mouse retinal cDNA libraries were screened using the T-box region of the *omb* gene as a hybridization probe to identify *omb*-related genes expressed in the retina. We examined expression of the three T-box genes encoding the transcription factors *Tbx2*, *Tbx3*, and *Tbx5* and found that each gene is expressed in an overlapping domain within the dorsal embryonic neural retina. As the retina differentiates, each gene is expressed in restricted and distinct subsets of retinal cells.

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Corresponding author: Jane C. Sowden, Developmental Biology Unit, Institute of Child Health, University College London, 30 Guilford Street, London, WC1N 1EH, UK. j.sowden@ich.ucl.ac.uk

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3095
Methods

In Situ Hybridization

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mouse embryos were obtained from matings of C57BL/6 x CBA mice. The day on which the vaginal plug was detected was designated embryonic day (E)0.5. Eyes from embryos at E10.5, E12.5, and E14.5 and from adult mice were analyzed. After informed consent and ethical permission had been sought and granted, human embryonic and fetal eye specimens were obtained from the Medical Research Council Tissue Bank and the Human Developmental Biology Resource, United Kingdom, according to the Polkinghorne Guidelines of the United Kingdom, which are in line with the tenets of the Declaration of Helsinki. Embryonic and fetal ages in weeks after conception were determined, either from hand and foot measurements, or, for older fetuses, by subtracting 2 weeks from the time since the last menstrual period. Tissues were fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C. Standard procedures were used to embed specimens in paraffin wax and for hybridization of 35S-radioisotope-labeled riboprobes to tissue sections.1,9 For human specimens, optimal hybridization signal was achieved, using cryosectioned tissue and nonradioactive digoxigenin-labeled riboprobes. After fixation, human eye specimens were placed in 20% sucrose in PBS for 24 hours, oriented dorsoventrally in optimal cutting temperature (OCT) compound and then flash frozen using isopentane and dry ice. Cryosections (10 μm) were mounted onto 3-amino propyltriethoxysilane (TESPA; Sigma, St. Louis, MO)-coated slides. In this study eyes at 6 weeks (n = 3), 8 to 9 weeks (n = 4, 12 to 15 weeks (n = 4), and 15 weeks (n = 2) were analyzed. Cryosections were hybridized with 1 ng/μl digoxigenin-labeled riboprobe, in hybridization buffer (1 mg/ml transfer [t]RNA, 50% formamide, 10% dextran sulfate, 15 μM each of the two complementary DNA (cDNA) strands, 1 mg/ml yeast RNA, 5 mM EDTA, 50% formamide, 10% dextran sulfate, 1 mM 3-(3-cholamidopropyl) dimethylammonio)-1-propanesulfonate (CHAPS), 1 mg/ml yeast RNA, 5 mM EDTA, 50 μg/ml heparin. Hybridizations were performed at 80°C and posthybridization washes were as follows: 5 minutes in solution 1 (50% formamide, 5% SSC, 0.1% Triton X-100, 0.5% 5-[3-cholamidopropyl] dimethylammonio)-2-hydroxy-1-propanesulfonate (CHAPS), 1 mg/ml yeast RNA, 5 mM EDTA, 50 μg/ml heparin. Hybridizations were performed at 80°C and posthybridization washes were as follows: 5 minutes in solution 1 (50% formamide, 5% SSC, 0.1% Triton X-100, 0.5% CHAPS); 5 minutes in three parts solution 1 to one part 2× SSC; 5 minutes in one part solution 1 to one part 2× SSC; 5 minutes in one part solution 1 to three parts 2× SSC; two times for 30 minutes each in 0.1% CHAPS and 2× SSC, and two times for 30 minutes each in 0.1% CHAPS and 0.2× SSC. All washes were performed at 65°C. Four E10.5 embryos were hybridized with each antisense and sense mouse probe in wholemount experiments.

The following plasmids were used to synthesize riboprobes for in situ experiments: (1) 498 bp of human TBX2 cDNA downstream of the T domain in pGEMZEE(+) (Promega, Southampton, UK); (2) 1.4 kb human TBX3 cDNA in pBluescript II-SK(–) (Stratagene, La Jolla, CA); (3) 2.4 kb human TBX5 cDNA in pBluescript II-SK(–); (4) 255 bp of the T-box region of mouse Tbx2 cDNA in pBluescript II-KS(--); (5) 255 bp of the T-box region of the mouse Tbx3 cDNA in pBluescript II-KS(–); (6) 1.1 kb of Tbx5 cDNA in pBluescript II-KS(–). Sense and antisense RNA transcripts were synthesized, using either SP6 polymerase (Roche) or T7 polymerase (Roche), and labeled with 35S-uridine triphosphate (UTP) or digoxigenin-UTP for hybridization to 8 μm tissue sections or wholemount embryos. To compare expression patterns of TBX2/Tbx2, TBX3/Tbx3, and TBX5/Tbx5, serial sections from human and mouse eye specimens were hybridized with respective probes. No signal was detected with the RNA sense probes. Embryos and sections were photographed with a photomicroscope (Diplan; Leica, Cambridge, UK) or a stereomicroscope (MZ12; Leica), respectively (Ekktachrome 64T film; Eastman Kodak, Rochester, NY), and the images were digitized on a scanner (FilmScan 200; Epson Seiko, Nagano, Japan) and assembled into figures on computer (Photoshop ver. 5.0; Adobe; San Diego, CA, and Powerpoint; Microsoft Corp. Redmond, WA).

Isolation of Mouse T-Box cDNAs

A 32P-labeled 1.8 kb BamHI fragment from the Drosophila omb cDNA, including the 600-bp T-box domain was used as a hybridization probe to screen a cDNA library in λZAP Express vector (Stratagene) prepared from neural retina and lens dissected from E15.5 mouse embryos; 5 × 105 plaque-forming units were hybridized overnight at reduced stringency at 52°C in a standard hybridization solution. Filters were washed twice in 0.5× SSC and 0.5% SDS at 52°C for 20 minutes before exposure to x-ray film at −80°C. pBK-CMV plasmids were excised from positive hybridizing plaques identified in tertiary screens and sequenced directly using plasmid primers flanking the insertion site and a dye terminator kit (Big Dye; Applied Biosystems, Foster City, CA). Of 14 clones analyzed, three contained T-box cDNAs. The other 10 clones that hybridized to the 1.8 kb omb probe did not hybridize to a smaller 300-bp omb probe, covering only the central T-box sequence, and encoded cDNAs for unrelated proteins, mouse lens α-crystallin, a protein of the major histocompatibility class II complex, and a protein elongation factor.

Degenerate PCR amplifications were performed using 1 μg mouse E15.5 retina and lens cDNA library in plasmid pBk-CMV. The degenerate primers span nucleotides encoding two conserved amino acid sequences. YIHPDSP and AVTAYQN, in the T-box of Drosophila omb and other T-box genes. PCR fragments of approximately 255 bp obtained after two 30-cycle rounds of amplification were gel purified using a gel extraction kit (QiaQuick; Qiagen, Crawley, UK) and subcloned into the pGem-T vector (Promega) according to the manufacturer’s instructions. Recombinant plasmids were sequenced directly.

Results

Identification of T-Box Genes Expressed in the E15.5 Mouse Retina

To isolate T-box genes, which are expressed in the developing retina and are related by sequence to the Drosophila omb gene, we used both conventional cDNA library screening and degenerate PCR amplifications. At E15.5 in the mouse, all six types of neuronal cell (rod, cone, horizontal, amacrine, bipolar, and ganglion) and Müller glial cells, which are characteristic of the mature neural retina, are developing and differentiating.1 A 32P-labeled cDNA probe that spans the T-box domain of the omb gene was used to screen a cDNA library prepared from E15.5 mouse neural retina and lens at low stringency. Four positive clones were identified and sequenced. Sequence comparisons indicated that three clones contained sequences identical with the Tbx2 cDNA previously isolated from E11.5 mouse embryos,11 and one contained sequence identical with Tbx5 cDNAs isolated from E8.5 mouse embryos and embryonic mouse limb cDNA libraries (GenBank...
Expression of *omb*-Related T-Box Genes in the Retina

By the end of the embryonic period of development (8 weeks), the developing T-box genes, *TBX2*, *TBX3*, and *TBX5*, are expressed across the developing eye (neural retina and lens). No other T-box cDNAs were detected. Across the 180-amino-acid (aa) region of the *omb* T-box domain (nucleotides [nt] 868-1408; Y16899), the mouse *Tbx2*, *Tbx3*, and *Tbx5* cDNAs share 66.9% nt (72.8% aa), 64.0% nt (72.6% aa) identity, respectively. The 5' untranslated leader sequence (compared with 418 bp in the *Tbx2* mRNA) is 129 bp long, and the 3' untranslated leader sequence is 9 bp long. (Figs. 1A, 1E, 1H) The *omb* cDNA contains a 129-bp untranslated region.

In the adult retina (Fig. 1T), expression of *Tbx2*, *Tbx3*, and *Tbx5* across the laminar axis of the developing neural retina was under way and the inner plexiform layer (IPL) had formed (Fig. 1N). *Tbx2* mRNA was most abundant in the developing ganglion cell layer (GCL; Fig. 1O). *Tbx3* mRNA, by contrast, was expressed exclusively in cells of the inner aspect of the dorsal neuroblastic retina (Fig. 1P, and low-magnification view, 1Q). The *Tbx5* expression domain began to narrow across the laminar axis at 8 to 9 weeks, and both were expressed across the width of the dorsal peripheral retina (Fig. 1G and data not shown).

By 12 to 13 weeks, the process of stratification of the neural retina was well underway and the inner plexiform layer had formed (Fig. 1N). *Tbx2* mRNA was most abundant in the developing ganglion cell layer (GCL; Fig. 1O). *Tbx3* mRNA, by contrast, was expressed exclusively in cells of the inner aspect of the dorsal neuroblastic retina (Fig. 1P, and low-magnification view, 1Q). The *Tbx5* expression domain began to narrow across the laminar axis at this stage and became restricted to newly born ganglion cells in the dorsal retina (Fig. 1J).

At 15 weeks, the GCL was clearly established, and the presumptive INL was visible because of the increasing stratification of the outer retina (Fig. 1Q). *Tbx2* mRNA was restricted to the GCL and to the developing INL, including cells at the inner aspect of the neuroblastic layer (Fig. 1R). *Tbx3* expression was no longer restricted across the dorsoventral retina axis and instead showed restriction across the laminar axis to cells of the nascent INL (Fig. 1S). At this stage, *Tbx5* mRNA could not be detected within the neural retina.

In the adult retina (Fig. 1T), expression of *Tbx2* was maintained in the GCL and could be detected within cells of the INL (Fig. 1U). No *Tbx2* expression was found in photoreceptor cells (Fig. 1V). Neither *Tbx3* nor *Tbx5* was detectable within the adult retina by in situ hybridization. However, RNA PCR amplification of adult neural retina samples indicated that low levels of *Tbx3* and *Tbx5* mRNA were in fact present (Fig. 2), suggesting that a small number of retinal cells may express these genes.
Expression of Tbx2, Tbx3, and Tbx5 in the Developing Mouse Retina

In situ hybridization of whole mount E10.5 mouse embryos (equivalent to ~4 weeks human gestation) was performed using digoxigenin-labeled Tbx2, Tbx3, and Tbx5 riboprobes. Tbx2 mRNA was detected in the dorsal hemisphere of the optic cup (Fig. 3A), consistent with previous reports.22 At this stage Tbx3 was also expressed within the dorsal portion of the optic cup, but at relatively low levels (Fig. 3B). Tbx5 mRNA was detected in the dorsal hemisphere of the optic cup simultaneously through the dorsal (superior) and ventral (inferior) retina, which at this stage consisted almost entirely of undifferentiated progenitor cells. The expression domains of the three genes were coincident (Figs. 3D–F). To compare these expression domains, adjacent coronal sections through the optic cup of E10.5 embryos were hybridized with the Tbx2, Tbx3, and Tbx5 35S-labeled riboprobes. The plane of section cut simultaneously through the dorsal (superior) and ventral (inferior) retina, which at this stage consisted almost entirely of undifferentiated progenitor cells. The expression domains of the three genes were coincident (Figs. 3D–F). The Tbx5 domain was tightly demarcated and lay within the broader Tbx2 domain, which extended more ventrally, whereas Tbx3 expression was indistinguishable at this stage from that of Tbx2 (Figs. 3D–F).

To examine variation in T-box gene expression across the retinal axes as retinal ganglion cells develop, transverse sections through the eye at E12.5 and E14.5 were analyzed by in situ hybridization (Fig. 4). By E12.5 in the mouse (equivalent to approximately 6 weeks of human development), cell differentiation in the neural retina was under way, and nerve fibers originating in the primitive ganglion cells were projecting toward the optic disc (Fig. 4A).23 Tbx2 and Tbx3 mRNAs were abundant throughout the dorsal hemisphere (Figs. 4B, 4C), but could not be detected in ventral retina (data not shown). Tbx5 mRNA was detected around the dorsoalmost third of the optic cup and was particularly abundant peripherally (Fig. 4D). The continued restriction of T-box gene expression to the dorsal retina at this stage of development and the tightly restricted domain of Tbx5 expression was consistent with the pattern of expression of the orthologous genes in the human retina.
By E14.5 (approximately 7–9 weeks of human development) the external and internal neuroblastic layers of the neural retina could be distinguished histologically (Fig. 4I). This time point is around the peak birth date of ganglion and amacrine cells.\(^1\)

\[\text{Tbx2}\] expression extended throughout the dorsal hemisphere. In transverse sections through the optic cup at the level of the optic nerve \[\text{Tbx2}\] mRNA was concentrated within the INB retina (Fig. 4E). The restriction to the INB retina was more marked dorsally (Fig. 4H) and was not apparent ventral to the optic nerve (Fig. 4K). In contrast, \[\text{Tbx3}\] mRNA levels were low within the central retina (Fig. 4F and data not shown). Strong \[\text{Tbx3}\] signal was only detected within the peripheral retina (Fig. 4F) in a dorsotemporal location. Here, its expression overlapped with \[\text{Tbx5}\] (Fig. 4G). Expression of \[\text{Tbx3}\] and \[\text{Tbx5}\] in the peripheral edge or ciliary margin of the retina extended across the width of the retina (Figs. 4F, 4G).

In addition to expression at the periphery, \[\text{Tbx5}\], similar to \[\text{Tbx2}\], was abundantly expressed at the location of the newly forming ganglion cells in the inner retina (Fig. 4G). The \[\text{Tbx5}\] expression pattern had a marked punctate appearance, suggesting only subsets of ganglion cells were labeled (Fig. 4G).

\[\text{Tbx5}\] labeling was detected in developing ganglion cells throughout the dorsal retinal hemisphere (Figs. 4G, 4J, 4M). The \[\text{Tbx5}\] expression domain had thus extended ventrally, compared with earlier stages (Fig. 4D).

At E14.5, \[\text{Tbx2}\] expression (Figs. 4E, 4H, 4K), but not \[\text{Tbx3}\] or \[\text{Tbx5}\] expression, was also abundant in the developing cornea and throughout the margins of the optic cup. At the optic cup margins, \[\text{Tbx2}\] expression was found both within the retina and in the overlying neural crest–derived mesenchyme that gives rise to the anterior segment of the mature eye. This observation is consistent with expression of \[\text{TBX2}\] mRNA within the developing cornea during human ocular development (Fig. 1F).

\[\text{Tbx2}\], but not \[\text{Tbx3}\] or \[\text{Tbx5}\] was also detected within the mesenchyme of the developing upper eyelids (Fig. 4L).

In the mature mouse retina (Fig. 4N), as in the human retina, \[\text{Tbx2}\] mRNA was most abundant within the GCL and INL, whereas the photoreceptor layer did not express \[\text{Tbx2}\] at significant levels (Fig. 4O). In comparison, \[\text{Tbx5}\] mRNA was not detected in the mature retina. \[\text{Tbx3}\] expression was de-

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**Figure 2.** PCR amplification of \[\text{TBX2}\], \[\text{TBX3}\], and \[\text{TBX5}\] mRNA from human retina. RNA PCR amplification of \[\text{TBX2}\], \[\text{TBX3}\], and \[\text{TBX5}\] mRNA from 10-, 11-, and 14-week human fetal eyes and adult neural retina (NR). \[\text{TBX2}\], \[\text{TBX3}\], and \[\text{TBX5}\] and ubiquitously expressed phosphoglucomutase, \[\text{PGM1}\]–specific primers, were used to amplify fragments of 209, 261, 295, and 417 bp, respectively. All primers are from exon sequences and PCR products span exon–intron boundaries. Thirty cycles of amplification were performed for each primer set to amplify aliquots of oligo dT-primed cDNA, under standard conditions. \[\text{Lane M}\]: molecular weight standard.

**Figure 3.** T-box gene expression in the embryonic mouse optic cup. In situ hybridization of wholemount E10.5 embryos hybridized with \[\text{Tbx2}\] (A), \[\text{Tbx3}\] (B), and \[\text{Tbx5}\] (C) digoxigenin-labeled riboprobes. Dotted line: plane of sections (D), (E), and (F). In situ hybridization of ocular sections from E10.5 embryos hybridized with \[\text{Tbx2}\] (D), \[\text{Tbx3}\] (E), and \[\text{Tbx5}\] (F) using radioactively labeled riboprobes. Arrows: expression of \[\text{Tbx2}\], \[\text{Tbx3}\], and \[\text{Tbx5}\] in dorsal hemisphere of the optic cup. Expression of \[\text{Tbx2}\] and \[\text{Tbx3}\] in the maxillary process (mx) and mandibular process (md) is also indicated. Scale bar, (A–C) 500 \(\mu\)m; (D–F) 200 \(\mu\)m.
ected within only a subset of cells of the INL (Fig. 4P), the location of these cells was consistent with the sites of expression of human TBX3 at 15 weeks.

**DISCUSSION**

In the present study at early stages of human and mouse development, three T-box genes, TBX2/Tbx2, TBX3/Tbx3, and TBX5/Tbx5, that are closely related to the *Drosophila omb* gene, were expressed in overlapping domains within the dorsal neural retina of the embryonic optic cup (Table 1). Recent reports of expression of the orthologous Tbx2, Tbx3, and Tbx5 genes within the dorsal optic cup of chick,24 *Xenopus* frog,25 and zebrafish embryos26,27 confirm a high level of evolutionary conservation of these spatial patterns of gene expression across the dorsoventral axis of the vertebrate eye.
Phylogenetic comparisons assign the vertebrate Tbx2, Tbx3, and Tbx5 genes to the same T-box gene subfamily as the invertebrate *omb* gene and suggest that these vertebrate genes arose by duplication of an ancient vertebrate *omb*-like gene sequence. In the eye imaginal disc of *Drosophila*, the *omb* gene does not display asymmetric expression. However, within leg and wing imaginal discs, *omb* expression is restricted to a dorsal compartment, suggesting some conservatism of function for *omb*-related genes in patterning of invertebrates and vertebrates. Other embryologic studies have demonstrated that Tbx2, Tbx3, and Tbx5 play important roles in vertebrate limb morphogenesis, and mutation of the human TBX3 and TBX5 genes both affect formation of the upper limbs. Defining the dorsosentral and nasotemporal axes of the neural retina is an essential step in development of the visual system. The most critical requirement for positional information across the developing retina is to provide coordinate identities to retinal ganglion cells and activate appropriate guidance molecules, because their axons project to topographic targets within the superior colliculus (optic tectum) and lateral geniculate nucleus. The early asymmetric expression patterns of the T-box genes reported here, Tbx2, Tbx3, and Tbx5, and by others in lower vertebrates, implicate all *omb*-related genes in patterning of invertebrates. The similarity between mouse and human expression patterns supports the use of the mouse as a model system for future functional studies. A number of transcription factors have been identified that show restricted patterns of expression across the laminar retinal axis during mouse development. Some of these factors have recently been shown to play critical roles in determining cell commitment and differentiation. The T-box gene expression is complex, in that it showed graduated expression in two dimensions, both across the retina and across the retinal layers (summarized in Table 1). These findings suggest a dual role for *omb*-related T-box genes in the human and mouse developing neural retina, in early dorsosventral patterning, and in the process of lamina- tion that accompanies the differentiation of retinal neuroblasts into the many cell types of the inner retina.

**References**

2. Drescher U, Kremoser C, Handwerker C, Loschinger J, Noda M, Bonhoeffer F. In vitro guidance of retinal ganglion cell axons by factors such as the T-box genes examined in this study. Only a handful of dorsally expressed transcription factors have been described previously. Understanding the interactions between these different classes of molecules is key to understanding the genetic control of development of retinal connections. In this respect it is interesting to note that in vitro both Tbx2 and Tbx3 function as transcriptional repressors, whereas other T-box proteins have transcriptional activator properties.

In the past year, two important studies have demonstrated the major role that asymmetrically expressed transcription factors play in activating the guidance molecules necessary for retinotopic projections. In chick embryos, misexpression of Tbx5 in the ventral retina leads to dorsalization of the ventral hemisphere and aberrant routing of the ventral projections, whereas misexpression of the enw-related homeobox gene Vax2 within the dorsal retina represses Tbx3 expression and causes targeting errors in dorsal projections.

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**Expression of omb-Related T-Box Genes in the Retina**

**Table 1. T-Box Gene Expression in the Developing Human and Mouse Retina**

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D. expression restricted to specific retinal layers, this is indicated as follows: INB, inner neuroblastic retina; GCL, ganglion cell layer; INL, inner nuclear layer.