Cellular Requirements for Building a Retinal Neuropil

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http://dx.doi.org/10.1016/j.celrep.2013.01.020

SUMMARY

How synaptic neuropil is formed within the CNS is poorly understood. The retinal inner plexiform layer (IPL) is positioned between the cell bodies of amacrine cells (ACs) and retinal ganglion cells (RGCs). It consists of bipolar cell (BC) axon terminals that synapse on the dendrites of ACs and RGCs intermingled with projections from Müller glia (MG). We examined whether any of these cellular processes are specifically required for the formation of the IPL. Using genetic and pharmacological strategies, we eliminated RGCs, ACs, and MG individually or in combination. Even in the absence of all of these partner cells, an IPL-like neuropil consisting of only BC axon terminals still forms, complete with presynaptic specializations and sublaminar organization. Previous studies have shown that an IPL can form in the complete absence of BCs; therefore, we conclude that neither presynaptic nor postsynaptic processes are individually essential for the formation of this synaptic neuropil.

INTRODUCTION

Synaptic neuropil is a major component of nervous systems, yet how it forms in specific places is not understood. It consists of intermingled axonal, dendritic, and glial processes, which form a dense array of specialized cellular connections. In many brain regions, neuropils form discrete layers beside or between collections of highly connected neurons. In the vertebrate retina (Figure 1A) there are two major layers of neuropil: the inner plexiform layer (IPL) and the outer plexiform layer (OPL). These neuropils, which consist of sets of cell-type-specific synapses, are completely devoid of cell bodies and thus are ideal models for studying the formation of synaptic neuropil. The IPL is where bipolar cell (BC) axons synapse onto the dendritic processes of retinal ganglion cells (RGCs) and the neurites of amacrine cells (ACs). The retina contains a single type of intrinsic glial cell, called Müller glia (MG). MG span the retina and send extensive processes into the plexiform layers (Figure 1A). Within the IPL, dendritic and axonal processes stratify within up to ten discrete sublaminae. This segregation relies on adhesive and repulsive guidance cues present in the IPL to guide them to their correct partner neurons (Matsuoka et al., 2011a, 2011b; Yamagata and Sanes, 2008, 2012; Yamagata et al., 2002). It is thought that for such interactions to occur, cues must be expressed either by synaptic partners or by other neurons or glia projecting within the neuropil (Timofeev et al., 2012; Matsuoka et al., 2011a). Therefore, it is important to identify the cell types that are critical for controlling the development of the neuropil layers.

One might hypothesize that the earliest-born cells, the RGCs, could organize a pre-IPL scaffold via their apically emerging dendrites. However, in ath5 (ath7) mutants, RGCs are absent, yet the IPL still forms (Kay et al., 2004). Similarly, BCs appear to be unnecessary for IPL formation, as the IPL still forms in Chx10; p27 (Green et al., 2003) and Math3; Mash1 (Tomita et al., 2000) double-mutant mice, which completely lack BCs. It has been suggested that ACs establish the IPL (Huberman et al., 2010; Kay et al., 2004). This suggestion was bolstered by a recent study that showed that extra misplaced IPLs formed when ACs failed to polarize their dendritic processes properly due to a loss of the protocadherin Fat3 (Deans et al., 2011). This work established the sufficiency of ACs for IPL formation, but whether they are necessary for such formation has not been yet tested. MG are also strong candidates for establishing the IPL, because retinal reaggregates form recognizable cell and plexiform layers when grown in the presence of a monolayer of MG (Willbold et al., 2000), but are disorganized and structurally inverted in the absence of MG (Layer et al., 1998).

To determine whether any particular component cells are essential for IPL-like neuropil formation, we selectively removed ACs, MG, and RGCs as individual cell types or in combination using mutants, morpholinos, and pharmacological inhibitors. Surprisingly, an IPL-like neuropil still formed in cellularly simplified retinas consisting of only BCs and photoreceptors (PRs). Remarkably, in this presynaptic-only neuropil, BC axons could still make presynaptic structures and display sublaminar organization of their axonal terminals. Together with previous findings, our results indicate that no single retinal cell type is critical for the formation of an IPL-like neuropil, and suggest that neuropil formation in the vertebrate CNS may result from the coordinated action of multiple autonomously stratifying cell types.
RESULTS

BC Basal Processes Retract from the Basal Surface of the Retina to Stratify Early within the Nascent IPL

To identify when BC axons first begin to enter the IPL, we labeled BCs either by vsx1:GFP or individually by the MAZe transgene (Collins et al., 2010), and imaged them by time-lapse confocal microscopy. BCs at early stages of their stratification usually have a thin distal basal process that extends to the basal lamina (Figures 1B and S1A; Movie S1). Later these distal processes retract, and BC axons branch within the IPL (arrows). This retraction is similar to that previously described for mouse BCs (Morgan et al., 2006), although this process happens much faster in zebrafish, taking 01:48 ± 00:13 (hr:min, mean ± SEM, n = 10 cells from four retinas) compared with 1 week in mice. The Kif5c560-based axon reporter (Distel et al., 2010; Jacobson et al., 2006; Randlett et al., 2011) labels these BC processes during the retraction and branching phase (Figure S1B, arrowheads) suggesting that these are indeed axonal processes.

If AC dendrites are critical for IPL formation, one would expect them to arborize into the nascent IPL before the BC axons do. To test this, we transplanted cells from transgenic donors containing the vsx1:GFP (to label BCs) and ptf1a::DsRed (to visualize all ACs and horizontal cells [HCs]) transgenes into unlabeled hosts (Godinho et al., 2005; Jusuf and Harris, 2009; Vittorino et al., 2009). These studies showed that BC axons began to collect among the cell bodies of differentiating ACs (arrows in Figure 1C; Movie S2) and appeared to part the displaced ACs among the cell bodies of differentiating ACs (arrows in Figure 1C; Movie S2) and appeared to part the displaced ACs (dACs) and normal ACs. This result supports the idea that BC axons arrive relatively early within the forming IPL. A similar imaging strategy, using the ath5::GAP-RFP transgenic to label RGCs and many ACs, demonstrated that BC axon terminals did not follow the emergence of an RFP-labeled plexus. Instead, the first BC axon branches and the RFP-labeled plexus became visible in the prospective IPL at approximately the same time (arrows in Figure 1D; Movie S3). Finally, we imaged the developing IPL using a fluorescent membrane marker expressed by all retinal cells. This allowed us to visualize BC axonal branches in an environment where all membranes were labeled (Figure S1C; Movie S4). Again, BC axons were visible within the earliest signs of IPL structure (arrows), indicating that BC axons are among the earliest colonizers of the IPL.

ACs Are Not Required for IPL Formation

Because AC dendrites do not obviously lead BC axons with respect to the time of arborization in the IPL, we wondered whether ACs are necessary for IPL formation. Ptf1a is a transcription factor that is expressed by all ACs and HCs in the zebrafish retina, and its disruption causes the respecification of these inhibitory neurons into excitatory ones (Jusuf et al., 2011; Jusuf and Harris, 2009). Ptf1a morpholinos alone do not completely remove all ACs (Jusuf et al., 2011), so we made use of a tiling mutant from the Zebrafish Mutation Project (ptf1a\textsuperscript{a126}). The ptf1a\textsuperscript{a126} mutant allele is a nonsense mutation that results in a truncation within the loop domain (Figure S2A). The ptf1a\textsuperscript{a126} embryos did not have an obvious morphological phenotype. Although they were markedly reduced in numbers, a substantial number of 5E11- and HuC/D-positive ACs remained in the

BC Partner Neurons Are Not Required for Neuropil Formation

Because neither of the two postsynaptic partners of BCs on their own appeared to be needed to form an IPL, we wondered whether they might act redundantly in this regard. If so, eliminating both RGCs and ACs simultaneously might lead to a failure of IPL formation. The ptf1a\textsuperscript{a126}/ptf1aMOs treatment, along with the ath5/lakritz mutant (in which RGCs are absent; Kay et al., 2001), provided the necessary tools to answer this question. vsx1:GFP/ath5\textsuperscript{a126};ptf1a\textsuperscript{a126}/ptf1aMOs embryos were fixed at 72 hpf and stained with HuC/D to determine the extent of AC/RGC loss (Figures S2D and S2E, arrowheads). Despite the loss of all neuronal postsynaptic partners, the BC axons still appeared to organize into an actin-rich neuropil, or IPL-like neuropil, positioned along the basal surface of the retina (Figures 2C and 2D). The simultaneous elimination of RGCs and ACs did not reduce the width of the IPL appreciably compared with the elimination of ACs alone (Figure 2E). Interestingly, the IPL was significantly thicker than WT after the elimination of RGCs alone. Because ACs are overproduced in this context (Kay et al., 2001), the thickness of the IPL in zebrafish may largely reflect the contribution of AC processes.

The sublaminar organization of the IPL is proposed to result from homophilic adhesion and guidance cue and receptor interactions between pre- and postsynaptic cell types (Matsuoka et al., 2011a, 2011b; Yamagata and Sanes, 2008, 2012; Yamagata et al., 2002). Therefore, we expected that IPL-like neuropil in the absence of ACs and RGCs might be completely devoid of any sublaminar organization. To test this, we used two transgenics that label different populations of BCs: Q16 and Q19. Q16 (nyx::mYFP) labels a population of BCs that stratify in the basal half of the IPL (Schroeter et al., 2006). We generated a transgenic based on the vsx1 promoter (Q19), which labels BCs that stratify in the apical half of the IPL. In WT retinas, imaging of the Q16;Q19 double transgenic revealed two bands of nonoverlapping terminals in the IPL-like neuropil (Figures 2F and 2H). These double transgenics were then injected with the ptf1aMOs and ath5MOs to eliminate RGCs and many ACs. Although the sublamination of the IPL was clearly less organized than in the WT, in this very thin IPL-like neuropil the typical apical/basal pattern was still obvious (Figures 2G and 2I) and highly significant (p = 3.0 \times 10^{-144}; Figure 2J). Sublamination of BC populations was also observed...
Figure 1. BC Axons Overshoot and Retract to Colonize the Nascent IPL

(A) Schematic showing the general organization of the vertebrate retina, including the neuropil layers, the IPL and OPL, and the retinal neurons and glia that will synapse within them.

(B) An individual BC labeled by MAGE:UAS:mYFP transgenes. The distal process extends to the basal surface (dashed line) of the retina. Branching into the IPL region can be seen, and the distal portion of the axon retracts to this point (arrow). At the same time, the apical process is also retracted from the apical surface of the retina.
MG Are Not Required for IPL Formation

If BCs, ACs, and RGCs (i.e., the full neuronal complement of the IPL) are each unnecessary for the formation of synaptic neuropil, there is only one other cellular component of the IPL that could be essential: the MG. Indeed, in the zebrafish mind bomb (mib) mutant, which lacks MG, retinal layering fails (Bernardos et al., 2005). The early interference with Notch signaling in this mutant, however, appears to compromise the differentiation of many other retinal cell types. To remove MG more selectively, we took a pharmacological approach. We administered the Notch pathway-blocking gamma-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester (DAPT) at 30–33 hpf, after neurogenesis had begun. Using this treatment regime, we found that retinal layering was intact, the IPL and OPL formed, and all of the neuronal cell types were still present and correctly positioned. vsx1:GFP-labeled BCs and ath5::GAP-RFP-labeled RGCs, ACs, HCs, and PRTs were all visible (Figure 4A). However, MG were completely absent throughout early development until at least 5 days postfertilization (dpf). This was confirmed by three separate immunohistochemical markers: anti-glutamine synthetase (anti-GS), anti-glial fibrillary acidic protein (anti-GFAP), and anti-cellular retinaldehyde-binding protein (anti-Crabp; Figures 4B and 4C), as well as the transgenic marker gnap::GFP (Figure 4D). To determine whether the IPL was still properly organized into sublamellar compartments, we again made use of the Q16 and Q19 transgenes. After the MG were removed, the BC axon terminals still separated into their proper distinct layers (arrow, Figure 4D). We also assayed for the presence of synaptic proteins by staining for the presynaptic vesicle marker SV2, the ribbon synapse component RibeyeA, and the postsynaptic density protein Maguk. All were present after the removal of MG at 72 hpf, suggesting that synapses were still able to form (Figure 4E).

In the Absence of All Other Component Cells, BC Axons Still Make an IPL-Like Neuropil

We have shown that a presynaptic IPL-like neuropil still forms after the removal of ACs, RGCs, and MG. However, again considering the potential for redundancy in this system, it is possible that any one of these cell types is sufficient on its own, and only the simultaneous removal of all three cell types will prevent IPL formation. Therefore, we removed all three of these cell types simultaneously by treating lak−/−:ptf1a−/−:ptf1aMO embryos in DAPT beginning at 33 hpf. Although ACs, RGCs, and MG were all absent from these retinas (Figure 5A), the BC axons still formed an actin-rich, neuropil-like layer along the basal surface of the retina (arrowhead, Figure 5B), and contained the presynaptic protein RibeyeA (arrowhead, Figure 5C). Remarkably, this BC-axon-only IPL-like layer also maintained overall sublamellar structure between two BC populations (Figure 5D).

DISCUSSION

Previous work has shown that the IPL forms in the absence of either BCs or RGCs, pointing to either ACs or MG as being essential for retinal layering and IPL formation (Bernardos et al., 2005; Huberman et al., 2010; Kay et al., 2004; Willbold et al., 2000). Indeed, it was suggested that BCs are passive players in IPL formation and are recruited to the preformed IPL through interactions with partner neurons (Huberman et al., 2010; Kay et al., 2004). In contrast to these models, we find that BC axons stratify within the nascent IPL, and that MG are not required for IPL formation. Not only are each of the individual cells discussed above essential for IPL formation; however, BCs are capable of forming a neuropil in the absence of MG, and MG are simultaneously eliminated. Although BC axons are capable of forming a neuropil autonomously and are present within the nascent IPL, they are themselves dispensable for IPL formation in mice (Green et al., 2003; Tomita et al., 2000). It is also interesting to note that in the absence of MGs, these IPL-like neuropil forms along the basal surface of the retina (rather than at its normal, more apical position), indicating that although these cells’ partner cells may not be necessary for IPL formation, they may play a role in the positioning of this neuropil layer.

(C) Transplantation scheme to create mosaic retinas with clones of vsx1:GFP-expressing BCs and ptf1a:DsRed-expressing ACs in an unlabeled host retina. At the onset of imaging, ptf1a:DsRed-expressing ACs and HC have migrated to the AC layer. However, a separation between the dACs and ACs is not apparent. Over time, the BC axons appear (arrows) and begin to stratify in between the ptf1a:DsRed-expressing cells. As these BC axons elaborate, the ACs are separated into displaced and nondisplaced populations that are part of the expanding IPL. Images are confocal reconstructions.

(D) Transplantation scheme to create mosaic embryos with vsx1:GFP-expressing BCs in a host retina where RGCs and ACs are labeled by ath5::GAP-RFP. BC axons (arrows) accumulate coincidentally with the appearance of the IPL, as shown by ath5::GAP-RFP-labeled RGCs and ACs (dashed line). Images represent maximum intensity projections of nine confocal slices. Time shown in h:min. Imaging begins at ~40 hpf. Scale bars = 10 μm. GCL, ganglion cell layer; INL, inner nuclear layer; NFL, neurofiber layer; OLM, outer limiting membrane; ONL, outer nuclear layer. See also Figure S1 and Movies S1, S2, and S3.
It is important to note that our treatment using mutants, morpholinos, and/or pharmacological inhibitors does not result in a loss of ACs; a phalloidin-rich IPL forms in the *ptf1a*−/−:*ptf1aMO* retina. Although it is unlikely, we cannot rule out the possibility that respecified cells retain properties reminiscent of their original fate (e.g., ACs with some RGC properties). Similarly, we were not able to completely rid the retina of ACs, but it seems very unlikely that the <1% of ACs that remain can drive the stratification of all other neurons across the retina.

Interestingly, in the absence of normal postsynaptic partners, BCs still appear to form presynaptic terminals replete with ribbons and synaptic vesicles abutting the processes of other BCs. This is reminiscent of previous reports that proper postsynaptic muscle targets are not necessary for presynaptic specializations in the *Drosophila* neuromuscular junction (Prokop et al., 1996), and that cultured spinal cord axons will form presynaptic specializations in contact with a polyornithine-coated bead (Peng et al., 1987). More surprisingly, the BC-only neuropil exhibited clear vestiges of sublamellar organization. BC axons that normally laminate in the apical half of the IPL still laminated...
apically to BC axons, which normally laminate basally. This observation highlights the importance of interactions among classes of presynaptic neurons, rather than simply among different types of neurons and glia, in setting up these segregated layers.

Because no single intrinsic retinal cell type is absolutely essential for retinal neuropil formation, and BCs can form a rudimentary neuropil autonomously, it seems reasonable to suspect that each of the three major neuronal types that contribute neurites to the IPL may also be able to do so autonomously. This is in line with studies in the embryonic Drosophila nerve cord, wherein it has been suggested that growing axons and dendrites are independently delivered to appropriate volumes of the developing neuropil by position-dependent guidance cues (Zlatic et al., 2009). If this explanation is correct, it will be important to discover which guidance cues are used to establish and position the retinal neuropil layers and how these cues are regulated.

Figure 3. BC Axons Form Presynaptic Structures without Postsynaptic Neurons

(A) WT ath5:GAP-RFP retinas stained with anti-HuC/D (to label ACs/RGCs) and anti-RibeyeA (to label ribbon synapses). Obvious punctate RibeyeA staining is visible in the IPL, whereas weak staining is seen in the BC cell bodies in the apical half of the INL and the OPL.

(B) When ACs and RGCs are absent in the ath5:GAP-RFP;ath5−/−;ptf1a−/−;ptf1aMO embryos, the weakly RibeyeA-staining BC cell bodies span the entire INL, and punctate RibeyeA staining is visible in the BC-only IPL. Scale bar in (A) and (B), 10 μm.

(C) Examples of vesicle-filled bouton-like structures (asterisks) containing ribbons near the retinal basal lamina of animals with severely reduced numbers of ACs and RGCs. Left: Higher magnification of the two such structures, presumed to be BC axonal boutons (arrowhead) shown in the inset. Right: Three consecutive sections (s1–s3) of processes in basally located neuropil in another animal. Arrows indicate ribbons juxtaposed to appositions with other processes, one of which can be another BC terminal (s1–s3).

(D and E) WT BC synapses exhibit readily apparent postsynaptic densities (thickenings, arrow) that are not seen in the mutant/morphant plexus (s1–s3).
EXPERIMENTAL PROCEDURES

Transgenic Lines and Constructs
Transgenic lines Tg(atoh7:gap43-mRFP1)cu2, Tg(vsx1:GFP)nnn5, Tg(MAZe), Tg(Ptf1a:DsRed), and Tg(nyx:Gal4-VP16)q16a;Tg(UAS:gap43-YFP)q16b have been described previously (Collins et al., 2010; Kimura et al., 2008; Schroeter et al., 2006; Vitorino et al., 2009; Zolessi et al., 2006) and are abbreviated here as ath5:GAP-RFP, vsx1:GFP, MAZe, ptf:DsRed, and Q16, respectively. The Vsx1:MCerulean (Q19) line was created using the upstream region of the Vsx1 gene (see Extended Experimental Procedures).

DAPT Treatment
Zebrafish embryos were treated with DAPT (50 μM) in 0.5% DMSO solution from 33 hpf to 3–4 dpf, and then fixed with 4% paraformaldehyde and cryoprotected in 30% sucrose in 1X PBS overnight prior to cryosectioning.

Figure 4. MG Are Not Required for Retinal Organization or Plexiform Layer Development
(A) ath5:GAP-RFP and vsx1:GFP show that all neural retinal cell types are present and correctly positioned in DMSO-treated control embryos and after DAPT treatment.
(B and C) Treatment with DAPT at 33 hpf completely removed MG in the retina. PKC staining is unperturbed by DAPT treatment, indicating BC differentiation is unaffected. However, DAPT-treated embryos show a complete loss of MG when stained with specific markers for GS, CRALBP, and GFAP when compared with DMSO controls.
(D) The IPL forms and sublaminates properly in the absence of MG. nyx:Gal4; UAS:MYFPQ16, and vsx1:MCeruleanQ19 fish show labeling of specific subsets of BCs that stratify in the apical (OFF) or basal (ON) domains of the IPL. Imaging of triple transgenics Q16;Q19;gfap:GFP treated with DAPT shows that the IPL still forms and BC axons sublamine properly (arrow). Lack of gfap:GFP signal confirms that there are no MG in the areas of proper sublaminarization.
(E) Pre- and postsynaptic markers remain in the plexiform layers lacking MG. Staining for the presynaptic markers RibeyeA and SV2 and the postsynaptic density protein Maguk (Mag) is unperturbed in embryos lacking MG at 72 hpf. Scale bars, 10 μm (A and D) and 20 μm (B, C, and E).
Figure 5. BC Axons Can Form a Sublaminated Neuropil in the Absence of Partner Neurons and Glia
(A–C) A neuropil layer (arrowhead) forms at the basal surface of a cellularly simplified retina ath5−/−;ptf1a−/−;ptf1aMOs treated with DAPT, lacking ACs, HCs, RGCs, and MG (A). The neuropil layer is actin rich (B) and contains RibeyeA positive staining at 72 hpf (C). (D) Gross sublaminar structure is maintained in cellularly simplified retinas, with Q19 apical to the PKC staining in the IPL-like layer. This sublamination was statistically significant (p = 6.22 × 10−44, n = 3 sections). Scale bars, 10 μm (A, D, and E) and 20 μm (B, C, F, H, and I).

REFERENCES
Bernardos, R.L., Lentz, S.I., Wolfe, M.S., and Raymond, P.A. (2005). Notch-Delta signaling is required for spatial patterning and Müller glia differentiation in the zebrafish retina. Dev. Biol. 278, 381–395.

Collins, R.T., Linker, C., and Lewis, J. (2010). MAZe: a tool for mosaic analysis of gene function in zebrafish. Nat. Methods 7, 219–223.

Deans, M.R., Krol, A., Abraira, V.E., Copley, C.O., Tucker, A.F., and Goodrich, L.V. (2011). Control of neuronal morphology by the atypical cadherin Fat3. Neuron 71, 820–832.

Distel, M., Hocking, J.C., Volkmann, K., and Köster, R.W. (2010). The centrosome neither persistently leads migration nor determines the site of axonogenesis in migrating neurons in vivo. J. Cell Biol. 191, 875–890.

Green, E.S., Stubbs, J.L., and Levine, E.M. (2003). Genetic rescue of cell number in a mouse model of microphthalmia: interactions between Chx10 and G1-phase cell cycle regulators. Development 130, 539–552.

Huberman, A.D., Clandinin, T.R., and Baier, H. (2010). Molecular and cellular mechanisms of lamina-specific axon targeting. Cold Spring Harb. Perspect. Biol. 2, a001743.

Jacobson, C., Schnapp, B., and Banker, G.A. (2006). A change in the selective translocation of the Kinesin-1 motor domain marks the initial specification of the axon. Neuron 49, 797–804.

Jusuf, P.R., and Harris, W.A. (2009). Ptf1a is expressed transiently in all types of amacrine cells in the embryonic zebrafish retina. Neural Dev. 4, 34.

Jusuf, P.R., Almeida, A.D., Randlett, O., Joubin, K., Poggi, L., and Harris, W.A. (2011). Origin and determination of inhibitory cell lineages in the vertebrate retina. J. Neurosci. 31, 2549–2562.
Kay, J.N., Finger-Baier, K.C., Roeser, T., Staab, W., and Baier, H. (2001). Retinal ganglion cell genesis requires lakritz, a Zebrafish atonal Homolog. Neuron 30, 725–736.

Kay, J.N., Roeser, T., Mumm, J.S., Godinho, L., Mrejeru, A., Wong, R.O., and Baier, H. (2004). Transient requirement for ganglion cells during assembly of retinal synaptic layers. Development 131, 1331–1342.

Kimura, Y., Satou, C., and Higashijima, S. (2008). V2a and V2b neurons are generated by the final divisions of pair-producing progenitors in the zebrafish spinal cord. Development 135, 3001–3005.

Layer, P.G., Rothermel, A., and Willbold, E. (1998). Inductive effects of the retinal pigmented epithelium (RPE) on histogenesis of the avian retina as revealed by retinospheroid technology. Semin. Cell Dev. Biol. 9, 257–262.

Matsuoka, R.L., Chivatakarn, O., Badea, T.C., Samuels, I.S., Cahill, H., Katayama, K., Kumar, S.R., Suto, F., Chédtal, A., Peachey, N.S., et al. (2011a). Class 5 transmembrane semaphorins control selective Mammalian retinal laminar formation and function. Neuron 71, 462–473.

Matsuoka, R.L., Nguyen-Ba-Charvet, K.T., Parray, A., Badea, T.C., Chédtal, A., and Kolodkin, A.L. (2011b). Transmembrane semaphorin signalling controls laminar stratification in the mammalian retina. Nature 470, 259–263.

Morgan, J.L., Dhingra, A., Vardi, N., and Wong, R.O. (2006). Axons and dendrites originate from neuroepithelial-like processes of retinal bipolar cells. Nat. Neurosci. 9, 85–92.

Peng, H.B., Markey, D.R., Muhlach, W.L., and Pollack, E.D. (1987). Development of presynaptic specializations induced by basic polypeptide-coated latex beads in spinal cord cultures. Synapse 1, 10–19.

Prokop, A., Landgraf, M., Rushton, E., Broadie, K., and Bate, M. (1996). Presynaptic development at the Drosophila neuromuscular junction: assembly and localization of presynaptic active zones. Neuron 17, 617–626.

Randlett, O., Poggi, L., Zolesii, F.R., and Harris, W.A. (2011). The oriented emergence of axons from retinal ganglion cells is directed by laminin contact in vivo. Neuron 70, 266–280.

Robu, M.E., Larson, J.D., Nasevicius, A., Beiraghi, S., Brenner, C., Farber, S.A., and Ekker, S.C. (2007). p53 activation by knockdown technologies. PLoS Genet. 3, e78.

Schroeter, E.H., Wong, R.O., and Gregg, R.G. (2006). In vivo development of retinal ON-bipolar cell axonal terminals visualized in nyx:MYFP transgenic zebrafish. Vis. Neurosci. 23, 833–843.

Timofeev, K., Joly, W., Hadjieconomou, D., and Salecker, I. (2012). Localized netrins act as positional cues to control layer-specific targeting of photoreceptor axons in Drosophila. Neuron 75, 80–93.

Tomita, K., Moriyoshi, K., Nakanishi, S., Guillemot, F., and Kageyama, R. (2000). Mammalian achaete-scute and atonal homologs regulate neuronal versus glial fate determination in the central nervous system. EMBO J. 19, 5460–5472.

Vitorino, M., Jusuf, P.R., Maurus, D., Kimura, Y., Higashijima, S., and Harris, W.A. (2009). Vsx2 in the zebrafish retina: restricted lineages through derepression. Neural Dev. 4, 14.

Willbold, E., Rothermel, A., Tomlison, S., and Layer, P.G. (2000). Müller glia cells reorganize reaggregating chicken retinal cells into correctly laminated in vitro retinas. Glia 29, 45–57.

Yamagata, M., and Sanes, J.R. (2008). Dscam and Sidekick proteins direct lamina-specific synaptic connections in vertebrate retina. Nature 451, 465–469.

Yamagata, M., and Sanes, J.R. (2012). Expanding the Ig superfamily code for laminar specificity in retina: expression and role of contactins. J. Neurosci. 32, 14402–14414.

Yamagata, M., Weiner, J.A., and Sanes, J.R. (2002). Sidekicks: synaptic adhesion molecules that promote lamina-specific connectivity in the retina. Cell 110, 649–660.

Zlatic, M., Li, F., Strigini, M., Grueber, W., and Bate, M. (2009). Positional cues in the Drosophila nerve cord: semaphorins pattern the dorso-ventral axis. PLoS Biol. 7, e1000135.

Zolesii, F.R., Poggi, L., Wilkinson, C.J., Chien, C.B., and Harris, W.A. (2006). Polarization and orientation of retinal ganglion cells in vivo. Neural Dev. 1, 2.