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Molecular mechanisms regulating synaptic specificity and retinal circuit formation

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Abstract

The central nervous system (CNS) is composed of precisely assembled circuits which support a variety of physiological functions and behaviors. These circuits include multiple subtypes of neurons with unique morphologies, electrical properties, and molecular identities. How these component parts are precisely wired-up has been a topic of great interest to the field of developmental neurobiology and has implications for our understanding of the etiology of many neurological disorders and mental illnesses. To date, many molecules involved in synaptic choice and specificity have been identified, including members of several families of cell-adhesion molecules (CAMs), which are cell-surface molecules that mediate cell-cell contacts and subsequent intracellular signaling. One favored hypothesis is that unique expression patterns of CAMs define specific neuronal subtype populations and determine compatible pre- and postsynaptic neuronal partners based on the expression of these unique CAMs. The mouse retina has served as a beautiful model for investigations into mammalian CAM interactions due to its well-defined neuronal subtypes and distinct circuits. Moreover, the retina is readily amenable to visualization of circuit organization and electrophysiological measurement of circuit function. The advent of recent genetic, genomic, and imaging technologies has opened the field up to large-scale, unbiased approaches for identification of new molecular determinants of synaptic specificity. Thus, building on the foundation of work reviewed here, we can expect rapid expansion of the field, harnessing the mouse retina as a model to understand the molecular basis for synaptic specificity and functional circuit assembly.

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AUTHOR CONTRIBUTIONS

Hannah Graham: Conceptualization; data curation; formal analysis; investigation; methodology; resources; validation; visualization; writing-original draft; writing-review and editing. **Xin Duan:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; supervision; validation; visualization; writing-original draft; writing-review and editing.

CONFLICT OF INTEREST

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Keywords

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1 INTRODUCTION

Neurons, the functional building blocks of the nervous system, are precisely organized into circuits in order to perform diverse computations to support behavior. How these circuits are assembled from diverse neuron types in the mammalian central nervous system (CNS) remains largely unknown. One critical feature of circuit assembly is synaptic specificity, which refers to a neuron's ability to identify and make connections with correct partners among a multitude of neighboring neurons, ensuring proper circuit function (Sanes & Yamagata, 2009; Yogev & Shen, 2014). The mechanisms underlying the development of synaptic specificity is a topic of great importance to the field of neuroscience, as these phenomena are ubiquitous and essential to the function of the brain and may shed light on a variety of disorders characterized by dysfunctional synapses and circuits (De Wit & Ghosh, 2016; Hirano & Takeichi, 2012; Kolodkin & Tessier-Lavigne, 2011; Koropouli & Kolodkin, 2014; Sudhof, 2017).

Studies in the mouse retina have been a major source of insight into mammalian synaptic specificity due to several unique advantages (Zhang, Kolodkin, Wong, & James, 2017). First, the retina's clear and stereotyped laminar organization makes histological observation of normal and aberrant circuit anatomy readily accessible. The five broader classes of retinal neurons are arranged in stereotyped and neatly laminated layers. Of these, there are three cellular layers, the outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL), and two synaptic layers, the outer plexiform layer (OPL) and the inner plexiform layer (IPL). Second, the retina is functionally accessible. Within just the retina, a tremendous amount of processing takes place, converting simple light detection into the sophisticated detection of a variety of features. This is possible due to a multitude of separate and parallel retinal circuits (Gollisch & Meister, 2010; Roska & Werblin, 2001; Seabrook, Burbridge, Crair, & Huberman, 2017; Wässle, 2004). The functions of these circuits can be determined, as light input can be precisely controlled, and the electrophysiological properties of different cells along the circuit can be measured directly. Third, retinal subtypes create evenly spaced mosaics. Therefore, determining whether a retinal type is a single and complete subtype can be determined in part by its pattern across the retina. Fourth, as a component of the CNS, it is likely that molecular and cellular strategies supporting synaptic specificity in the retina are generalizable to other regions of the CNS, many of which also contain a variety of neuronal types and subtypes that adhere to a similarly laminated organization (Sanes & Yamagata, 1999; Williams, de Wit, & Ghosh, 2010).

An essential prerequisite to the study of synaptic specificity is an understanding of the cellular players involved. In other words, a basic “parts list” of the types of neurons that make up a given circuit is principal to understanding how these parts wire together and what each part contributes to the circuit (Zeng & Sanes, 2017). Within the five broad classes of retinal neuron, photoreceptors (PRs), horizontal cells (HCs), bipolar cells (BCs), amacrine cells (ACs), and retinal ganglion cells (RGCs), there are at least ~70 subtypes based on morphological and functional criteria (Sanes & Masland, 2015). Specifically, it is now estimated that there are 15 subtypes of BCs, ~45 subtypes of ACs, and ~50 subtypes of RGCs (Baden et al., 2016; Helmstaedter et al., 2013; Rheaume et al., 2018; Shekhar et al., 2016). Additionally, genetic access to many neuron subtypes is now possible due to the generation of transgenic mouse lines (Huberman et al., 2009; Kim, Zhang, Yamagata, Meister, & Sanes, 2008; Siegert et al., 2009; Yonehara et al., 2009). A traditional method of subtype classification is neuronal morphology, which in turn affects a neuron’s intrinsic electrophysiological properties. Furthermore, the location of a neuron’s dendritic and axonal processes determines, in part, its pre- and postsynaptic partners, and consequently, the circuits in which it participates (Sanes & Zipursky, 2010). In the retina, neurons target their arborizations to one or a few of several lamina within the OPL or IPL, and synaptic partners are found directly apposed within the same lamina. Mounting evidence supports the notion that laminar targeting is largely determined by the expression of CAMs, which are molecular mediators for neuron–neuron interactions. In summary, neuronal morphology and molecular composition are intimately related to each other, to the identity of a neuron, and to its ability to make specific synaptic connections and assemble into the proper circuits. Extending the same wiring principles to the brain, the axons of different subtypes of RGCs exit the eye and make long-range connections with one or several different brain areas, such as dorsal lateral geniculate (dLGN), superficial superior colliculus (sSC) and suprachiasmatic nucleus (SCN) to drive distinct behaviors (Berson, Dunn, & Takao, 2002; Dhande et al., 2013; Kay et al., 2011; Martersteck et al., 2017).

In the search for molecular determinants that mediate neuronal subtype specific wiring, it is rational to start with extant transgenic mouse lines, as described above. These mice allow researchers to visualize the morphologies of and gain genetic access to specific retinal neuronal subtypes during development. Furthermore, recent transcriptomic profiling approaches using these transgenic lines have been used to register specific CAMs to individual retinal neuron subtypes. For these reasons, the mouse retina is the first mammalian CNS region approaching complete genetic subtype classification, an otherwise impossible undertaking using conventional approaches. In many cases, this direct link between neuronal subtype identity and specific CAM expressions has been extended to reveal a role for CAMs in synaptic specificity and circuit properties (De La Huerta, Kim, Voinescu, & Sanes, 2012; Duan, Krishnaswamy, De La Huerta, & Sanes, 2014; Kay et al., 2011; Kim et al., 2008; Liu & Sanes, 2017; Südhof, 2018; Yogev & Shen, 2014; Yonehara et al., 2008). With the increasing accessibility of powerful, unbiased sequencing and functional approaches, the retina will undoubtedly continue to be a fertile discovery platform for molecules underlying synaptic specificity and circuit assembly. Recent advances in our understanding of the molecular underpinnings of retinal synaptic specificity, and the link from neuronal subtypes to circuit functions will be the primary focus of this review.

2 MOLECULAR MECHANISMS GOVERNING NEURITE MORPHOGENESIS AND NEURONAL SPACING

The size and shape of a neuron's arborization constrains the size of its receptive field and the number and density of synapses its makes. Consequently, regulation of neurite morphology is critical for a neuron to fulfill its role within a circuit. Taking RGCs as an example, multiple subtypes of RGCs send their dendrites into the IPL, creating the need for several kinds of organizational strategies. First, a single RGC often symmetrically maximizes its dendritic coverage area in order to evenly sample from across its receptive field. This relies on a strategy for recognizing and repulsing a neuron's own dendrites. Second, a single RGC subtype must be evenly spaced across the entire retina, without oversampling, in order to evenly sample across the visual field. Recognition and repulsion of a neuron's own dendrites is separable from interactions between dendrites of neurons of the same subtype (homotypic neurons). These recognitions are often termed "self versus non-self" discrimination (Zipursky & Grueber, 2013). The laminar organization of the retina confines neurites into a compact two-dimensional space in one of two plexiform layers, the OPL or IPL. This structure provides a simple framework for studying certain themes of contact-mediated recognition and repulsion. These themes are informative to our general understanding of neuronal development throughout the mammalian nervous system, as the same strategies and molecular players are often reused in different contexts. In this section, we will focus on molecules that contribute to shaping three aspects of retinal neuron morphogenesis, namely, self versus non-self discrimination and homotypic interactions, dendritic symmetry, and somatic spacing. There are many factors that contribute to retinal neuron morphogenesis that are beyond the scope of this review, but have been elegantly described elsewhere (Lefebvre, Sanes, & Kay, 2015).

As briefly mentioned above, a single retinal neuron employs molecular strategies to avoid excessive dendritic crossovers between its own dendrites. A classic example of a molecule involved in self vs. non-self discrimination is Down's syndrome cell adhesion molecule (DSCAM). DSCAM and its related proteins are cell surface and soluble proteins that are involved in cell-cell recognition. *Drosophila Dscam1* is stochastically alternatively spliced into ~38,000 isoforms, which enables extraordinary molecular diversity and allows for the dendrites of the same cell to distinguish its own dendrites from the dendrites of neighboring cells (Schmucker et al., 2000). A neuron's own dendrites, which express the same repertoire of *Dscam1* isoforms, are repelled from one another, a phenomenon known as dendritic self-repulsion, whereas dendrites of neighboring cells, which stochastically express a different repertoire of isoforms, are free to interact (Hattori et al., 2007; Matthews et al., 2007; Schmucker et al., 2000; Soba et al., 2007). In the mammalian retina, starburst amacrine cells (SACs) have been the model neuron for studies of self versus non-self discrimination. Unlike many other retinal neurons, SACs overlap extensively, forming a dense dendritic plexus in the IPL which is critical in generating direction-selectivity in the retina (Morrie & Feller, 2018). In vertebrates, *Dscam* does not contain complex alternative exons to support alternative splicing, meaning SACs must rely on a different strategy for self vs. non-self discrimination. Although sharing no sequence homology to *Dscam*, the γ subcluster of protocadherin (*Pcdhg*) in mice was found to be utilized in a similar strategy. Rather than

alternative splicing, alternative promoter choice and isomer multimerization allows neurons to stochastically express ~10,000 unique combinations of the 22 *Pcdhg* isoforms on its cell surface, each of which binds only homophilically, resulting in repulsion (Schreiner & Weiner, 2010; Tasic et al., 2002). Genetic deletion of all 22 *Pcdhg* genes from SACs results in loss of dendritic self-avoidance, characterized by excessive crossovers and fasciculation between the neurites of a single SAC and neighboring SACs, as though the recognition of "self" becomes indistinguishable from "non-self" homotypic neighbors (Lefebvre, Kostadinov, Chen, Maniatis, & Sanes, 2012). Supporting this hypothesis, SACs lacking all *Pcdhgs* also frequently form autapses (Kostadinov & Sanes, 2015). Linking neurite morphogenesis to circuit function, Kostadinov also showed that this loss of dendritic self-avoidance results in defects in the directly downstream direction-selective circuits. Intriguingly, they also found that selective expression of a single isoform of *Pcdhg* in SACs is sufficient to rescue defects in dendritic self-avoidance. However, overlap with neighboring SACs is also significantly reduced. Thus, *Pcdhg* isoform expression is required for dendritic self-repulsion, and *Pcdhg* diversity is required for homotypic overlap. In other words, SACs appear to rely on diverse and random expression of *Pcdhg* isoforms to distinguish between their own neurites, which are repelled, and those of their neighbors, which are not. Deletion of the related *Pcdha* cluster has no effect on SAC dendritic morphology. However, the combined deletion of *Pcdhg* and *Pcdha* causes more severe defects than *Pcdhg* deletion alone, indicating that these two clusters may functionally interact (Ing-Esteves et al., 2018). Applying this finding to elsewhere in the CNS, Lefebvre also showed that the elaborate dendrites of cerebellar Purkinje cells become disorganized and exhibit many dendritic crossings after deletion of *Pcdhgs*. Ing-Esteves also showed that this Purkinje cell phenotype is exacerbated in double *Pcdhg* and *Pcdha* mutants. Additionally, in the olfactory bulb, combined loss of *Pcdha*, *Pcdhb*, and *Pcdhg*, but not any one alone, results in severe arborization and self-avoidance defects of olfactory sensory neuron axons (Mountoufaris et al., 2017). Most recently, 25 mouse lines with deletions of various combinations of *Pcdhgs* were generated using CRISPR/Cas9 in order to perform detailed *Pcdhg* gene cluster analysis (Garrett et al., 2019). Garrett discovered that a single isoform, *PcdhgC4*, is necessary and sufficient for neuronal survival. In other words, isoform diversity is not essential for neuronal survival.

While the functional advantage of symmetrical dendrites is fairly straight-forward, some neurons have asymmetrical dendrites. In these cases, researchers have sought the molecular underpinnings of these irregular morphologies and their impact on cellular and circuit function. The most prominent example in the retina is J-RGCs, which have a characteristic asymmetrical dendritic arbor, which in turn confers direction-selectivity by a SAC-independent mechanism. Indeed, asymmetric J-RGCs that are found in the ventral- and dorsal-most parts of the retina do not exhibit directions-electivity (Liu & Sanes, 2017). These RGCs highly express junctional adhesion molecule B (JAM-B), a transmembrane protein with two extracellular immunoglobulin-like domains (Marie-Laure et al., 2011), and are marked by the transgenic JAM-B-CreER line (Kim et al., 2008). The progressive development of the asymmetrical dendritic arbors of J-RGCs appears to be due to a combination of asymmetric dendritic growth and selective pruning of dendrites pointing in other directions (Liu & Sanes, 2017). Liu also provided compelling evidence for the role of

non-cell autonomous mechanisms in shaping J-RGC arbors. It was found that local ablation of RGCs results in reorientation of J-RGCs neighboring the ablation site. Loss of JAM-B itself in J-RGCs results in a minor decrease in dendrite crossovers, indicating that JAM-B might be required to promote adhesive interactions between a neuron's own dendrites (Liu & Sanes, 2017). While much focus has been paid to molecules required for dendritic self-avoidance, the example of JAM-B illustrates that repulsive forces could be counterbalanced by additional adhesive forces. The precise nature of the molecular mechanisms underlying J-RGC dendritic asymmetry is still unknown, but do not appear to be directly related to JAM-B.

It has now been well established that retinal neurons form mosaic patterns in the horizontal plane of the retina, and that the cell bodies of a given subtype of neuron are more evenly spaced than would be expected by a random distribution (Wassle & Riemann, 1978). Mosaicism and tiling ensure uniform coverage of the retina and even sampling of the entire visual field for each parallel stream of processing (Wässle, 2004). The mosaics of different types of neurons are independent of one another, suggesting that unique molecular cues must mediate homotypic repulsion for different neuronal subtypes. Mosaics give rise to an exclusion zone, in which an area around a cell of a given subtype is unlikely to include another cell of the same subtype. Even spacing of dendritic arbors, rather than cell bodies, is a related phenomenon referred to as tiling. Tiling is often described in terms of coverage factor, where a coverage factor of one represents no dendritic overlap, and coverage factors greater than one indicate partial overlap. For example, as discussed earlier, SACs have significant homotypic dendritic overlap, so their coverage factor is approximately 30 (Sun et al., 2013). In a recent study, the protein AMIGO2 was discovered to scale the size of SAC and rod BC dendritic arbors (Florentina Soto et al., 2019). AMIGO2 is a homophilic cell-surface protein with extracellular leucine-rich repeats (LRRs) that was found to be expressed by both SACs and rod BCs in early postnatal development. Knockout of *Amigo2* resulted in selective expansion of the dendritic arbors of both of these cell types, thereby increasing the coverage factor. Nevertheless, SACs remained functionally connected with their postsynaptic partners, direction-selective ganglion cells. Interestingly, the direction-selectivity of this circuit was actually enhanced.

The literature suggests that dendritic tiling may be a transient mechanism by which to establish proper mosaic spacing of cell bodies. In support of this model, it was found that the somata of HCs are randomly spaced early in development, but form mosaics after dendritic tiling is established (Huckfeldt et al., 2009). Huckfeldt also found that laser ablation of a single HC during early development induces neighboring HCs to reposition their somas and expand their dendritic territory to fill the gap, presumably in response to relief from a repulsive cue. Interestingly, in mutant mouse lines in which only a small percentage of RGCs are present, RGCs are evenly spaced and their dendritic field sizes are normal (Lin, Wang, & Masland, 2004). Thus, for RGCs, evidence suggests that normal dendritic morphology and spacing are genetically hardwired, and homotypic interactions act as cues for additional fine-tuning. In another example, two related transmembrane proteins, MEGF10 and MEGF11, are found to be expressed in SACs, whose somata reside in both the INL and GCL, and HCs during early postnatal development. Loss of these molecules results in aberrant mosaic spacing of these cell types (Kay, Chu, & Sanes, 2012). Thus, MEGF10

and MEGF11 are required for mosaicism in SACs and HCs. In SACs, these molecules were found to be critical for transient homotypic interactions between SAC processes as they migrate to the nascent IPL and oriented their processes. It was later shown that MEGF10 mediates homotypic contacts between newborn migrating SACs, and these contacts are critical for subsequent recruitment and innervation of SAC circuit partners (Ray et al., 2018). Reuse of two cell-surface proteins to mediate three independent mosaics is possible in structures such as the retina that have clearly laminated layers, preventing the processes of these three populations from occupying the same space.

Additional molecules that regulate neurite morphology, such as semaphorins, plexins, and vertebrate DSCAMs, also play extensive roles in laminar targeting and will be discussed in subsequent sections.

3 SYNAPTIC SPECIFICITY IN THE OUTER PLEXIFORM LAYER

In the mouse retina, rods and two types of cones are the primary light sensors, and their cell bodies make up the ONL. These three types of photoreceptors (PRs) make synapses in the OPL onto the processes of HCs and BCs, whose cell bodies reside in the INL. The tripartite synapse between PRs, HCs, and BCs constitutes the first step of signal processing in the visual system (Figure 1). HCs are a single neuron type that primarily provide lateral feedback inhibition to modulate the signal from PRs to BCs. On the other hand, PRs connect to one or several of 15 types of BCs (Behrens, Schubert, Haverkamp, Euler, & Berens, 2016; Shekhar et al., 2016; Wässle, Puller, Müller, & Haverkamp, 2009). Based on electrical recordings and anatomical evidence, the connectome of the outer retina has been partially described (Behrens et al., 2016; Wässle et al., 2009). Rod BCs tend to contact approximately 35 rod PRs, whereas cone BCs tend to make between 3 and 8 cone PR contacts. The rod and cone PR pathways display a high degree of overlap. For example, rod PRs contact several kinds of cone BCs, and both M- and S-cone PRs contact rod BCs. Additionally, aside from two exceptions, cone BCs are achromatic, in that they contact both M- and S-cone PRs. (Behrens et al., 2016). The number and kinds of BCs that a given rod or cone PR forms synapses with is highly specific and determines the properties of the visual field that is propagated forth (Behrens et al., 2016; Dunn & Wong, 2012). Thus, synaptic specificity in the OPL is critical to the initial divergence of parallel processing streams in the retina. In this section, molecules involved in the development, function, and maintenance of synapses in the OPL will be discussed.

Semaphorins and plexins are conserved mediators of short-range signaling underlying synaptic specificity in many developmental systems. Semaphorins are secreted and membrane-bound guidance cue proteins that have been shown to be critical in processes including axon guidance, cell migration, and dendritic arborization in the nervous system (Tran, Kolodkin, & Bharadwaj, 2007). Plexins are the most prominent of the semaphorin receptors. In the developing outer retina, SEMA6A and PLEXA4 are both expressed throughout HC neurites. It was shown that interactions between these molecules mediates HC laminar arborization and ensures correct formation of synapses between HCs, BCs, and PRs (Matsuoka et al., 2012). In mice with mutations in either *Sema6A* or *PlexA4*, HC neurites that are normally confined to the OPL are ectopically extended into the ONL

(Figure 2). These ectopic neurites do not form synapses with BCs or PRs, indicating that the laminar targeting of these cells is unaffected. However, Matsuoka found that rod PR ribbon synapses of *PlexA4* mutants often contain only one HC process instead of two, although this does not significantly affect synaptic function, as assessed by ERG. Matsuoka also found that HCs display defects in dendritic self-avoidance, but mosaic spacing is unaltered. These findings indicate that SEMA6A-PLEXA4 signaling is critical for HC lamination, rod PR synaptic terminal invasion, and neurite morphology.

Another molecule, the leucine-rich repeat-containing protein netrin-G ligand 2 (NGL-2) was found to play a role in OPL synapse development, as well as in mediating the functional connectivity between HCs and rods (Soto, Watkins, Johnson, Schottler, & Kerschensteiner, 2013). NGL-2 is expressed selectively on the axon tips of developing HCs at the site of HC synapses onto rod PRs. The axons of HCs in *Ngl-2* mutant mice extend ectopically into the ONL, similar to the phenotype observed in *Sema6A-PlexA4* mutants. HC axon territories are also expanded, but axons form fewer synapses with rod PRs, resulting in defective signal transmission between rod PRs and rod BCs. In contrast, HC dendrites develop normally and connections with cones are preserved. In a follow-up study, Soto used AAV deletion and overexpression of *Ngl-2* in individual HCs. First, this study confirmed that deletion of *Ngl-2* from HCs causes expansion of HC axon territories and results in fewer synapses with rod PRs. This phenotype was observed even when *Ngl-2* was deleted in HCs of young adult mice. Second, AAV-induced expression of *Ngl-2* in *Ngl-2* knockout mice restores normal HC morphology and synapse numbers, and overexpression in wildtype mice results in abnormally high numbers of HC synapses (Soto, Zhao, & Kerschensteiner, 2018). These results indicate that NGL-2 is critical for axon targeting and morphology, synapse formation, and synapse maintenance in the HC-rod circuit. NETRIN-G2 is a known interaction partner for NGL-2 that is expressed by PRs, so Soto suggested that the interaction between these two molecules might mediate the transsynaptic interaction between HCs and PRs. Most recently, another leucine-rich repeat-containing protein, LRRTM4, was found to play a critical role in rod BC synapse formation (Sinha et al., 2020). LRRTM4 is a trans-synaptic adhesion protein that is concentrated at GABAergic synapses onto rod BCs. Knockout of *Lrrtm4* causes a reduction in $GABA_A$ and $GABA_C$ clustering on rod BCs, and thus disruption of presynaptic inhibition. Additionally, rod BC terminals display impairments in dyad formation, instead forming monads and triads.

Synaptic Cell Adhesion Molecule 1 (SYNCAM1) has been well studied in the formation of a synapses in the brain, and has also been found to be important in rod to HC connectivity in the retina (Ribic, Liu, Crair, & Biederer, 2014). SYNCAM1 is highly expressed on the terminal membranes of rods in a developmentally regulated manner, and in knockout mice, rods form fewer synapses and rod-mediated light responses are disrupted. Additionally, ectopic HC processes in the ONL, like those in *Sema6A-PlexA4* and *Ngl-2* mutant mice, are observed, also likely contributing to the impaired rod light-transduction pathway (Figure 2). While studies in the brain have shown that SYNCAM1 elevation is required to maintain increased synapse number and plays a role in synaptic plasticity, there is no evidence yet for these nondevelopmental roles in the retina (Ribic, Crair, & Biederer, 2019; Robbins et al., 2010)

In a large scale screen for cell surface molecules mediating OPL development, a role for a noncanonical WNT signaling pathway in rods was identified (Sarin et al., 2018). Using RNA-Seq, the authors found that WNT ligands, WNT5A and WNT5B are selectively expressed in rod BCs during development. CRISPR mutagenesis of either or both of these molecules results in a duplicated ectopic OPL located in the ONL (Figure 2). This ectopic OPL is composed of processes of rods, cones, rod BCs, cone BCs, and HCs. Using cell-type specific expression of CAS9, Sarin then showed that noncanonical WNT signaling from rod BCs to rods, through RYK, FZD4, and FZD5 co-receptors is necessary for proper OPL development. The cellular mechanism by which WNT5 is required for OPL development is still unclear. Sarin proposed three possibilities. First, WNT5 might act to directly promote growth of rod terminals towards the OPL. Second, it might stabilize interactions between rods and their targets within the OPL. Third, misplaced rod axons might be repelled by WNT5 released from rod BCs, promoting directional translocation of these ectopic rods to the correct location.

It is likely that in cases described above in which defects in circuit functioning are observed, these defects are most likely a secondary consequence to ectopic neurite targeting. Other molecules, such as ELFN1, have been identified as critical for OPL circuit integration and synapse function without these major anatomical defects. A proteomic screen for binding partners of MGLUR6, a postsynaptic receptor at the synapses between photoreceptors and ON-BCs, identified ELFN1, another CAM of the leucine-rich repeat family, as the highest hit (Cao et al., 2015). ELFN1 is expressed selectively on rod axon terminals and was found to form direct trans-synaptic contacts with ON-BC mGluR6 dendrites. Consequently, loss of ELFN1 prevents the formation of this specific synapse and causes functional defects in the rod visual pathway (Figure 3).

Studies concerning synaptic specificity in the OPL are emerging in aging and degenerative disease models. For example, studies on homeostatic remodeling mechanisms following PR death provide particular insight into the etiology and progression of diseases such as age-related macular degeneration (Care et al., 2019; Dunn, 2015). In the retinas of old mice, for example, HC and BC neurites aberrantly extend into the ONL where they form ectopic synapses. Deletion of the serine/threonine kinase LKB1 throughout the retina or in rods alone induces similar ectopic HC and BC sprouts and synapses in young mice, as well as the consequent retraction of rod axons (Samuel et al., 2014). This was found to be mediated through disruptions in LKB1-AMPK signaling (Figure 4). This study identified LKB1-AMPK signaling as a regulator of synaptic aging in the OPL and underscored its role in maintaining normal laminar positioning in the developed retina.

4 SYNAPTIC SPECIFICITY IN THE INNER PLEXIFORM LAYER

Generally, IPL synapse formation follows the same general rules of CNS synaptogenesis. The limited number of neuronal subtypes and their clear functional readouts offer careful examinations of the roles of molecules in each and every step of synaptogenesis. BC axons project from the ONL to the IPL, forming synapses with RGCs and ACs, which have cell bodies in the GCL and both the GCL and INL, respectively (Figure 5). Similar to HCs in the OPL, ACs act primarily to modulate the signal from BC to RGC in the IPL. The IPL is

separated into five major laminae (S1-S5) in which particular combinations of BCs, ACs, and RGCs form synapses. This lamination is stereotyped, in that cells of a given type will preferentially arborize in one or a few of these laminae. Moreover, it is governed by combinations of molecules involved in repulsive or adhesive forces. The retina uses lamination as a strategy to maximize wiring of correct pre- and postsynaptic partners and prevent wiring of inappropriate partners (Zhang et al., 2017). Consequently, laminae are circuit-specific, and the lamina in which a cell arborizes offers clues as to the kind of visual information the cell processes. For example, the processes of cells that are tuned to decrements in light reside in S1-S2 (OFF laminae), and those tuned to increments in light reside in S3-S5 (ON laminae). Moreover, ON-OFF Direction-Selective Ganglion Cells (ooDSGCs) are responsive to both ON and OFF stimuli and have bistratified dendrites in S2 and S4. After targeting the correct lamina, additional molecules involved in local synaptic targeting decisions come into play for the “fine-tuning” or circuit assembly. These various molecules will be discussed in the subsequent paragraphs.

To understand the principles underlying IPL selective choice, researchers started by searching for immunoglobulin superfamily (IgSF) proteins with restricted sublaminar expression. In the chick retina, DSCAM and DSCAM-Like (DSCAML) are expressed in nonoverlapping subsets retinal neurons, are present in distinct IPL sublaminae, and mediate homophilic adhesion (Yamagata & Sanes, 2008). Deletion of these proteins revealed that these molecules are necessary to direct lamina-specific arborization for distinct circuits. Moreover, their ectopic expression is sufficient to redirect arborizations to distinct, ectopic laminae. In the developing mouse retina, DSCAM is expressed by a subset of ACs and most RGCs, and both DSCAM and DSCAML function through homotypic repulsion (Fuerst et al., 2009; Fuerst, Koizumi, Masland, & Burgess, 2008). Mice with mutations in either of these genes have excessively fasciculated RGC, AC, and rod BC dendrites, as well as clumped cell bodies. Nevertheless, these aberrant processes still stratify in the correct IPL laminae and form functional connections with the appropriate partners. Thus, in the mouse studies, but not those in chick, DSCAMs are dispensable for synaptic specificity but are required for mosaic patterning of cell bodies and dendritic morphology. Furthermore, in the chick, DSCAMs mediated homophilic adhesive interactions, whereas in the mouse, the DSCAMs mediate dendritic self-avoidance. This may reflect species specific differences or a context-dependence of function, with different downstream signaling pathways potentially mediated by the same recognition molecules. Indeed, in the chick retina, it was found that DSCAMs interact with members of the membrane-associated guanylate kinase with inverted orientation (MAGI) subfamily to direct laminar specificity (Yamagata & Sanes, 2010). MAGIs are synaptic scaffolding proteins, and like DSCAMs, are expressed in subsets of retinal neurons. Through different combinatorial expression patterns of these various proteins, different specificities and downstream effects are possible. Lastly, without alternative splicing, the mechanism by which vertebrate DSCAMs mediate dendritic self-avoidance is clearly distinct from that in *Drosophila*. Fuerst hypothesized that vertebrate DSCAMs may passively prevent adhesion, and that their loss unmasks intrinsic adhesive cues. Indeed, there is evidence that through distinct functional interactions with different cadherins and protocadherins, vertebrate DSCAM may act as a general “nonstick” signal,

thereby promoting self-avoidance, not through repulsion, but through masking of cell-type specific adhesive cues (Garrett, Khalil, Walton, & Burgess, 2018).

Two more IgSF proteins, Sidekick-1 (SDK-1) and Sidekick-2 (SDK-2), were also found to mediate homophilic adhesion in the chick retina (Yamagata & Sanes, 2008; Yamagata, Weiner, Sanes, & Louis, 2002). Each SDK was found to be expressed at the synaptic site of presynaptic and postsynaptic neurons that project to the same IPL sublaminae. Furthermore, ectopic expression of these molecules is sufficient to redirect the processes of other neurons to these sublaminae. In mice, SDK-2 localizes at the synapses of W3B-RGCs, also known as local edge detectors, and a presynaptic partner, VG3-ACs (Krishnaswamy, Yamagata, Duan, Hong, & Sanes, 2015). The authors then showed that homophilic interactions mediated by SDK-2 are necessary for the development of this selective synaptic connection and the proper functioning of W3B-RGCs (Figure 7).

In addition to the SDKs and DSCAMs, another closely related group of IgSF molecules known as Contactins (CNTNs) have been found to be important for retinal laminar specificity (Yamagata & Sanes, 2012). CNTN1–5 are largely expressed by nonoverlapping subsets of retinal neurons in the INL and GCL of the developing chick retina and are concentrated in discrete bands in the INL. Neurons that express CNTN2 normally laminate in S2 and S4. However, knockdown of CNTN2 results in diffuse arborization throughout the IPL. Furthermore, ectopic expression of CNTN2 redirects the arbors of other neurons to S2 and S4. Thus, similar to the DSCAMs and Sidekicks, CNTN2 is necessary and sufficient for laminar targeting of neurites in the chick retina, and in vitro studies suggest that this is mediated through homophilic adhesion.

It should be noted that certain cell-recognition molecules are used in both the IPL and OPL, a strategy that is possible only due to anatomical separation of these plexiform layers. As in the OPL, Semaphorin-Plexin signaling is involved in synaptic specificity and laminar targeting in the IPL. In the developing mouse inner retina, both SEMA6A and its receptor PLEXA4 are expressed in complementary cell types and mediate laminar specificity through heterophilic interactions (Matsuoka et al., 2011a). PLEXA4 is present in S1-S2, whereas SEMA6A is present in S3-S5. Mice with mutations in either of these genes exhibit misdirected neurite arborizations for several classes of retinal neurons without coincident defects in other aspects of dendritic morphology, such as dendritic self-avoidance. For example, dopaminergic AC arborizations that are typically confined to S1, form ectopic arborizations in S4-S5. These findings suggest that SEMA6A serves as a repulsive barrier for neuronal processes expressing the PLEXA4 receptor. Further studies have found that SEMA6A-PLEXA2 signaling mediates SAC dendritic morphology and laminar stratification, as well as the functioning of the RGCs that SACs synapse on (Sun et al., 2013). In early postnatal development, ON SACs, which are sensitive to light increments, express both SEMA6A and PLEXA2, another receptor for SEMA6A, whereas OFF SACs express only PLEXA2. In mice with mutations in these genes, the arborizations of ON and OFF SACs fail to segregate, and ON SAC dendritic symmetry is disrupted due to defects in dendritic self-avoidance (Figure 6). Consequently, ooDSGCs, which receive directional input from both ON and OFF SACs, have defective direction-selectivity in response to ON stimuli. Therefore, repulsive heterophilic interactions mediated by these proteins are

necessary for the functioning of the ON-OFF direction-selective circuitry. Another study found that SEMA5A and SEMA5B provide repulsive guidance cues to the developing arborizations of AC and RGC types expressing PLEXA1 and PLEXA3 (Matsuoka et al., 2011b). In the absence of either of these ligands or receptors, ACs and RGCs project ectopic neurites into the INL without defects in mosaic patterning or dendritic tiling. Stratification in the OFF laminae of the IPL is most severely affected, and consequently, RGC responsivity to decrements in light are disrupted.

Using a high-throughput, receptor-ligand biochemical screen of candidate proteins for retinal laminar specificity, a novel interaction between Fibronectin Leucine-Rich Transmembrane (FLRT) and Uncoordinated5 (UNC5) proteins was identified (Visser et al., 2015). To validate these findings, the authors found that in vivo, FLRT1–3 and UNC5A,C,D exhibit differential laminar-restricted expression in the developing IPL. FLRT2 and UNC5C are expressed in complementary IPL sublaminae, and FLRT2 is expressed in a pair of synaptic partners, SACs and ooDSGCs. Both of these cell types are repelled by UNC5C in vitro. Thus, Visser and colleagues suggested that FLRT2-UNC5C interactions may support the proper targeting of SAC and ooDSGC neurites to the same laminae, promoting their preferential connection. This screen identified an additional 24 previously unreported interactions, such as those between Semaphorins, Neuropilins, and Plexins which remain to be validated.

Fully elucidating the IPL connectivity map and linking synaptic specificity between the neuronal subtypes directly back to their molecular expression and circuit function is nearly within the reach of retinal neurobiologists. At least for one family of CAMs, the cadherins (CDHs), this has been achieved on a smaller scale within the ON-OFF direction-selectivity circuits. CDHs are calcium-dependent cell surface glycoproteins that mediate homophilic adhesion and are known to be expressed in complex, combinatorial patterns throughout the nervous system (Hirano & Takeichi, 2012). Among the >100 members of the CDH superfamily, the most studied are the type I and II classical CDHs. In the retina, the classical CDHs, CDH8 and CDH9, are expressed selectively by two BC subtypes during retinal development (Duan et al., 2014). BC2s express CDH8 and project to the OFF laminae of the IPL, providing input about decrements in light to ooDSGCs. BC5s express CDH9 and project to the ON sublaminae, providing input about increments in light. Deletion of *Cdh8* or *Cdh9* results in BC2 or BC5 axon targeting to both ON and OFF laminae, and consequently, functional deficits in immediately downstream ooDSGCs. Conversely, Duan found that ectopic expression of these molecules is sufficient to instruct laminar targeting. In other words, overexpression of CDH9 in BC2s redirects arbors to the normal location of BC5 arbors, and vice versa (Figure 6). This misdirected targeting is possible even in a subtype of AC that normally expresses neither CDH8 nor CDH9. Of great functional relevance, misdirected BC2 and BC5 cells maintain their distinct circuit roles and are able to propagate OFF and ON stimuli, respectively, to their nonstandard partners. These findings suggest that CDH8 and CDH9 function to bias BC axonal arborization in the IPL. Unexpectedly, this appears to be mediated through heterophilic, rather than homophilic, interactions. The heterophilic partners have not been identified but may include different CDHs or other cell-surface recognition molecules. Interestingly, CDH6 may also play a role in the ON-OFF direction-selectivity circuits, as it is selectively expressed in ooDSGCs that

respond to ventral or dorsal motion (Kay et al., 2011). Although CDH10 is also expressed in these ooDSGCs, deletion of neither *Cdh6*, *Cdh10*, nor a combination of the two results in any structural or physiological defects in these cells (Duan et al., 2018). However, CDH9 is ectopically expressed in these cells in double *Cdh6* and *Cdh10* mutants. Duan found that deletion of *Cdh6*, *Cdh9*, and *Cdh10* together results in a drastically abnormal structural and physiological phenotype. Ventral- and dorsal- ooDSGCs in triple mutants have diffuse and variably distributed dendrites throughout the IPL, rather than tight cofasciculation with SACs in S2 and S4 (Figure 7). The direction-selectivity of these neurons is also greatly reduced. In parallel, ooDSGCs with preference for nasal motion may rely on combined expression of CDH7 and CDH18 for appropriate wiring with SACs. These studies illustrate an amazing division of labor between different related molecules of the same family to direct laminar and synaptic specificity of several different retinal neuron types, and support the hypothesis of a combinatorial “adhesive code” underlying specific neuronal connectivity (Redies & Takeichi, 1996). Using the same set of genetic reagents, others have demonstrated similar combinatorial cadherin “adhesive codes” in the developing hippocampus and spinal cord, suggesting this may be a heavily utilized strategy throughout the CNS (Basu et al., 2017; Dewitz, Duan, & Zampieri, 2019).

Notably, these circuit-based analyses can offer interesting mechanistic insights into human disorders. One such an example is FRMD7, a member of the FERM domain protein family, which functions in cytoskeletal reorganization, and is specifically expressed in SACs (Yonehara et al., 2016). In humans, mutations in FRMD7 results in congenital nystagmus, in which the horizontal optokinetic reflex (OKR) is lost. In mice, FRDM7 hypomorphic mutants also displayed OKR loss, accompanied by loss of horizontal, but not Vertical direction-selective responses in ooDSGCs. Yonehara determined that the source of this defect is loss of asymmetric connectivity between SACs and ooDSGCs. Thus, the primary defect in FRMD7 hypomorphic mice is in horizontal direction-selectivity within the retina, which is then propagated forth to the brain centers required for OKR, namely the NOT and DTN of the accessory optic system. Yonehara suggested that FRDM7 may be part of the molecular machinery involved in sensing or differential sorting of molecules along the horizontal axis, supporting the asymmetrical wiring between SACs and ooDSGCs of different directions.

Early work on key transcriptional factors for RGC specification suggested that they influence cellular identity, morphology, and function by driving differential expression of cell surface proteins, such as the ones detailed above. These would then go on to mediate further intercellular interactions. The POU-domain containing BRN3 transcription factors play a variety of roles in the development of retinal neurons, including their morphology. For example, deletion of BRN3A leads to an increase in the ratio of bistratified to monostratified RGCs (Badea, Cahill, Ecker, Hattar, & Nathans, 2009). However, the molecules downstream of BRN3A that mediate this laminar choice are yet to be determined (Sajgo et al., 2017). At individual RGC subtype resolution, a few key transcriptional factors have emerged. One such an example is the transcriptional regulator SATB1 for ooDSGCs. SATB1 was found to influence ooDSGC laminar choice via regulation of CNTN5 (Peng et al., 2017). In *Satb1* mutant mice, ooDSGCs, which normally laminate in S2 and S4, preferentially direct their arborizations to S2 and consequently, have defective ON responses. This phenotype is

partially recapitulated in ooDSGCs with loss of CNTN5, the expression of which was found to be promoted by SATB1. CNTN5 and its co-receptor CASPR4 are normally expressed by both ooDSGCs and ON SACs and ON BCs, suggesting that homophilic interactions between ooDSGC ON dendrites and ON SACs and BCs stabilize ooDSGC arbors. Similarly, T-box brain 1 (TBR1) was identified as a regulator of cell-surface molecules that direct laminar targeting of four RGC types with dendrites in the outer third of the IPL (Liu et al., 2018). Focusing on J-RGCs, the authors showed that TBR1 acts through two downstream cell-surface molecules, CDH8 and SORCS3, to target J-RGC dendrites to S1 of the IPL. Moreover, ectopic expression of TBR1 is sufficient to mistarget the dendrites of other neuronal types to the outer IPL. A key next step in our understanding of synaptic specificity will be determining the transcriptional players' upstream and molecular effectors downstream from the cell-surface molecules that have been so far identified.

5 RETINOFUGAL PROJECTIONS

The final step in the light-transduction pathway within the retina is the exit of RGC axons from the optic nerve head. These axons each carry information computed by one of multiple parallel circuits composed of upstream retinal neurons. It is estimated that there are ~46 retinorecipient nuclei, supporting both image-forming and non-image forming functions, although it is estimated that 90% of RGC axons terminate in the superficial superior colliculus (sSC) (Dhande, Stafford, Lim, & Huberman, 2015). These axons travel along the optic tract, project either ipsilaterally or contralaterally at the optic chiasm, and then form synapses in various visual system nuclei (Zhang et al., 2017). The ability of axons from about 50 subtypes of RGCs to make stereotyped and accurate long-range projections to reach one or several of 46 postsynaptic targets is an impressive feat, mediated by selective expression of a variety of cell surface molecules on RGC axons that confer differential responses to diverse extracellular cues.

The optic chiasm is an anatomical fork in the road, serving as the first point of axon divergence. Here axons will decide to either cross the midline and project to the contralateral hemisphere or turn back ipsilaterally. The degree of chiasm crossing varies in different species, depending on their use of binocular vision. For example, in highly binocular humans, 40% of axons are uncrossed, whereas in mice, only 3–5% are uncrossed (Petros, Rebsam, & Mason, 2008). The transcription factor ZIC2 has been well characterized in left–right asymmetry of the body plan, and it has also been shown to specify ipsilateral turning RGCs by regulating responses to repulsive cues in the chiasm (Herrera et al., 2003), likely by promoting and inhibiting expression of selective cell surface molecules to mediate these responses. Among the many axon guidance cues and receptors, EPHB1-EPHRIN-B2 signaling has emerged as a major determinant of the ipsilateral turn of axons at the chiasm. The EPHB1 receptor is expressed in regions of the retina that give rise to ipsilateral projections, and *EphB1* null mice exhibit a significant reduction in ipsilateral projections (Williams et al., 2003). EPHRIN-B2, a ligand for EPHB1, is expressed in the chiasm during ipsilateral turning. in vitro studies suggest that the nature of this interaction is repellant. Their similar temporal and spatial localizations during development suggest that ZIC2 may directly or indirectly regulate the expression of EPHB1. Indeed, it was shown that ZIC2 expression in RGCs is necessary and sufficient for ipsilateral turning, and acts in part

through upregulation of EPHB1 (García-Frigola, Carreres, Vegar, Mason, & Herrera, 2008). Interestingly, Garcia-Frigola found that even in the absence of EPHB1, ZIC2 can induce some ipsilateral turning. This suggests that independent ZIC2-related mechanisms are also in play. Additionally, ZIC2 delivery to retinal explants is sufficient to induce upregulation of EPHB1 expression at nonipsilateral turning RGC growth cones (Lee, Petros, & Mason, 2008).

Additional notable examples of molecular determinants of chiasm choice include Sonic Hedgehog (SHH), NRCAM, and neuropilin 1 (NRP1). SHH is secreted by contralateral RGC axons at the optic chiasm and repels ipsilateral RGC axons through the SHH receptors BOC and SMO (Peng et al., 2018). Additionally, NRCAM is expressed in retinal regions that give rise to contralateral projections as well as the chiasm, and *Nrcam* null mice have a significant reduction in contralateral projections (Williams et al., 2006). Lastly, the NRP1 receptor expressed by contralateral projecting axons promotes midline crossing in response to the chemoattractant guidance signal VEGF164 present in the chiasm (Erskine et al., 2017). The presence of both ipsilateral and contralateral projection-promoting factors at the chiasm argues against the long-standing hypothesis that midline crossing is a default behavior for RGC axons and indicates that a combination of multiple push and pull strategies may dictate axon crossing.

After making a midline decision, axons must defasciculate from the optic tract at the right place and terminate in the proper retinorecipient targets. Within the optic tract, ipsilateral and contralateral RGC axons are fasciculated and segregated by midline crossing behavior. Specifically, ipsilateral axons are offset laterally from contralateral axons. Interestingly, ipsilateral axons in *EphB1* null mice, which exhibit erroneous decussation choices at the chiasm, remain positioned lateral to contralateral axons; however, they appear less fasciculated than in wildtype (Sitko, Kuwajima, & Mason, 2018). These misrouted axons have been shown to continue to target the ipsilateral zone of the dLGN (Rebsam, Petros, & Mason, 2009). Therefore, loss of guidance cue signals important for proper midline crossing do not necessarily prevent type-specific segregation and proper targeting of axons. The extracellular matrix molecule, reelin was found to be required in axons of intrinsically photosensitive RGCs (ipRGCs) to properly innervate the ventral lateral geniculate nucleus (vLGN) and intergeniculate leaflet (Su et al., 2011). Reelin deficient ipRGC axons are instead mistargeted to adjacent nonretinorecipient targets. In another interesting study, CDH6 was found to be expressed in multiple retinorecipient targets during late embryonic and early postnatal development, when RGC axons select their targets in the brain. Multiple RGC types that also express CDH6, including ipRGCs, selectively innervate these targets, suggesting that CDH6 mediates pre- and postsynaptic matching (Osterhout et al., 2011).

Several retinorecipient targets have precise topology and innervation architecture, indicating that even within a given target, the termination of axons is specified. For example, especially in the sSC and dorsal lateral geniculate nucleus (dLGN), different RGC types have characteristic innervation patterns (Hong, Kim, & Sanes, 2011; Kay et al., 2011; Martersteck et al., 2017). However, the molecular determinants of the specific terminations of subtypes of RGCs remains to be identified in both of these structures. In particular, it is still unknown

how ipsilateral and contralateral projecting RGC axons selectively innervate superficial or deep layers of the SC, or the core and shell of the dLGN, respectively.

6 CONCLUSIONS AND OUTLOOK

In summary, paramount to the proper functioning of the retina and any other region of the nervous system, is the ability to form functional circuits. This in turn is dependent on synaptic specificity, in which a neuron is able to identify and connect with the appropriate pre- and postsynaptic partners. The ability of a neuron to do this is contingent on the concurrence of several events. First, the arborizations of a neuron must be in the right place at the right time. The spatial and temporal control of neurite arborization is reliant on the presence of the proper combination of molecules on the cell surface, which can sense permissive and prohibitive environments, and properly guide neurites during development. The spatial organization of a neuron's dendritic field, including factors such as symmetry, density, and size, in turn, determine the electrophysiological properties of the cell and put physical constraints on the number and density of synapses that can be made. In the retina, it is critical that dendritic and axonal arborizations are targeted to the correct laminae in either the OPL or IPL. In addition to the retina, retinorecipient areas, and many other brain regions, rely on lamination to place pre- and postsynaptic partners in direct apposition, facilitating proper wiring and vision-guided behaviors (Cheng et al., 2010; Hong et al., 2011; Huberman et al., 2009; Kim, Zhang, Meister, & Sanes, 2010). RGCs must send long-range projections to the correct retinorecipient areas in the brain, an undertaking that relies upon many of the same molecules used for their dendritic targeting (Osterhout, Stafford, Nguyen, Yoshihara, & Huberman, 2015; Sun et al., 2015).

Secondly, once in the right location, neurons must be able to pick the correct synaptic partners among many incorrect choices. This is dependent on the expression on complementary recognition molecules that facilitate homophilic or heterophilic adhesion. The ability of the 100–150 different retinal cell types to achieve synaptic specificity appears to be reliant on the combinatorial expression of these molecules, allowing the same molecule to be reused in various cell types, but due to co-expression of other recognition molecules or association with alternate downstream effectors, drive distinct specificities in each. Accordingly, cadherin and Immunoglobulin superfamily (IgSF) "codes" have been proposed to drive retinal synaptic specificity (Duan et al., 2014; Redies & Takeichi, 1996; Yamagata & Sanes, 2008; Yamagata & Sanes, 2012). This review described several of these molecules, but there are undoubtedly many more that are yet to be identified.

There are several nonmolecular determinants of synaptic specificity that were not the focus of this review; however, they are important to make note of. The patterning of connectivity throughout the nervous system is often believed to follow an activity-dependent, competition-based model, in that less active inputs are preferentially eliminated in lieu of more active ones. In the retina, through selective genetic silencing of neurotransmission, it was found that neurotransmission does indeed facilitate synapse formation, but that cells form synapses autonomously, rather than in a competitive manner (Kerschensteiner, Morgan, Parker, Lewis, & Wong, 2009; Okawa et al., 2014a; Okawa et al., 2014b). Additionally, selective ablation of the dominant input to a class of ON RGC during development induces

circuit-level homeostatic plasticity. In other words, new synaptic partners are recruited, and connections with minor partners are increased (Tien, Soto, & Kerschensteiner, 2017).

The expression of recognition molecules that determine laminar targeting and synaptic choice is largely driven by different transcriptional programs and is part of the genetic identity of a given neuronal type. Thus, morphology and molecular content of a neuron are inextricably linked, and determine identity, connectivity, and function. Most distinct neuronal types in the retina will be defined by a combination of several molecular markers, many of which will be cell surface molecules. An understanding of additional molecular markers for subtypes and subsets of neurons will facilitate the classification of new and existing retinal neuronal subtypes and their function. Additionally, they will provide genetic entry points for isolation and manipulation (Hartl, Krebs, Jüttner, Roska, & Schübeler, 2017; Krieger, Qiao, Rousso, Sanes, & Meister, 2017; Rousso et al., 2016; Siegert et al., 2009; Zeng & Sanes, 2017). The advent of single cell sequencing technologies will undoubtedly facilitate this process (Laboissonniere et al., 2019; Macosko et al., 2015; Rheaume et al., 2018; Shekhar et al., 2016). However, post-hoc in vivo validation will be required, including functional assays, such as calcium imaging with retinal subtype resolution (Baden et al., 2016; Liu et al., 2018). Although some headway has been made in terms of connectomic mapping both within the retina and between the retina and brain, there is still much more to be learned (Helmstaedter et al., 2013; Martersteck et al., 2017). Ultimately, in combining these efforts with the new molecular profiling technologies, retinal subtypes can be tied back to connectivity maps, and the molecules instructing this connectivity can be identified and validated. The retina is an ideal discovery platform for these molecules, and it is likely that findings will be generalizable throughout the nervous system.

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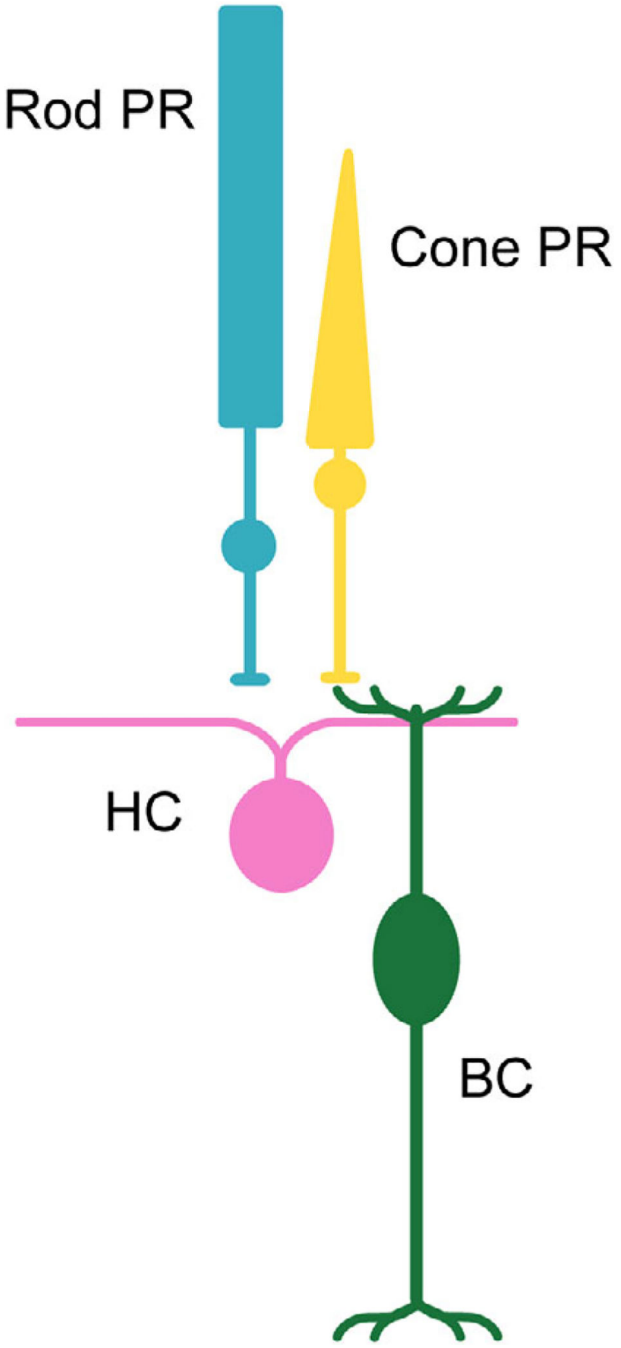

FIGURE 1.
Diagram of neuron types in the outer retina. Rod (turquoise) and cone (yellow) photoreceptors (PRs) receive light input and signal to postsynaptic horizontal cells (HCs) (pink) and bipolar cells (BCS) (green)

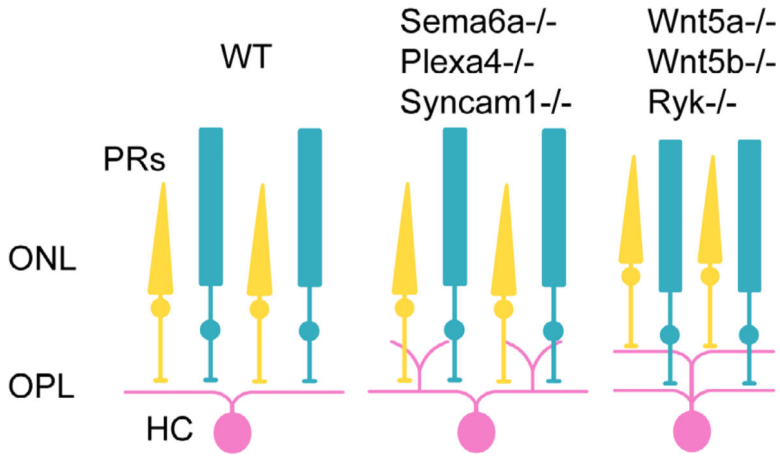

FIGURE 2.

Laminar targeting in the OPL. Wildtype horizontal cells (HCs) and photoreceptors (PRs) are depicted on the left. Matsuoka et al. found that HCs express SEMA6A and its receptor PLEXA4, and that loss of either results in ectopic neurites in the outer nuclear layer. Ribic et al., found that loss of SYNCAM1 results in a very similar HC dendritic morphological defect (center). Sarin et al. found that rod bipolar cell (BC) expression of WNT ligands signal to rods through the receptor RYK to mediate synapse formation between these cell types. Loss of these molecules results in ectopic plexiform formation, composed of neurites from PRs, BCs, and HCs (right)

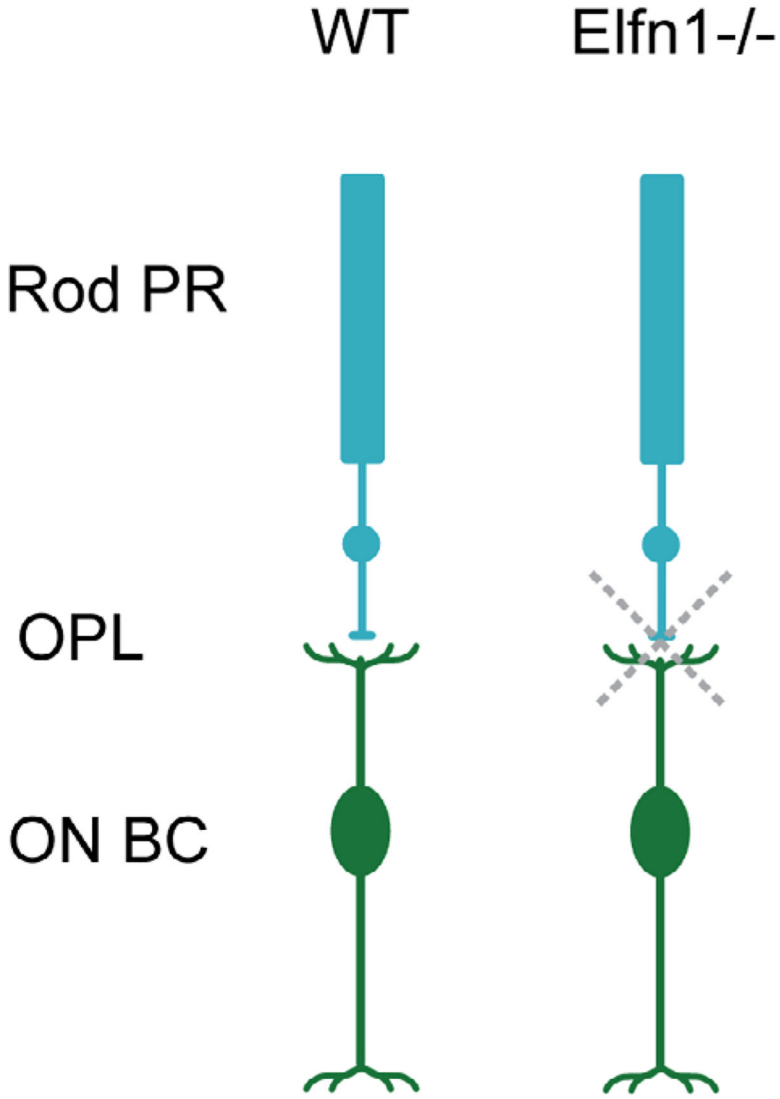

FIGURE 3.

Synapse formation in the OPL. Wildtype rod photoreceptors (PRs) and bipolar cells (BCs) are depicted on the left. Cao et al. identified ELFN1 as a rod-expressed, direct binding partner of ON bipolar cell MGLUR6. Loss of ELFN1 prevents proper synapse formation between these two neurons (right)

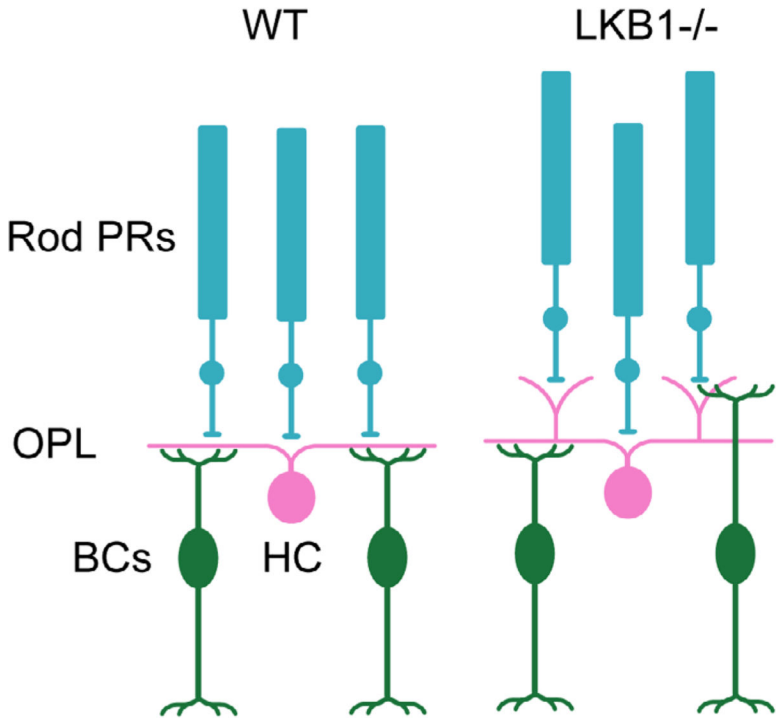

FIGURE 4.

Synaptic maintenance in the OPL. Wildtype horizontal cells (HCs), rod photoreceptors (PRs), and bipolar cells (BCs) are depicted on the left. Age-related loss of synaptic integrity results in ectopic neurites and synapses in the outer nuclear layer, due to retraction of rod axons. Samuel et al. found that loss of LKB1-AMPK signaling induces similar changes in the retinas of young mice, implicating these molecules in the maintenance of normal synaptic architecture in the outer plexiform layer (right)

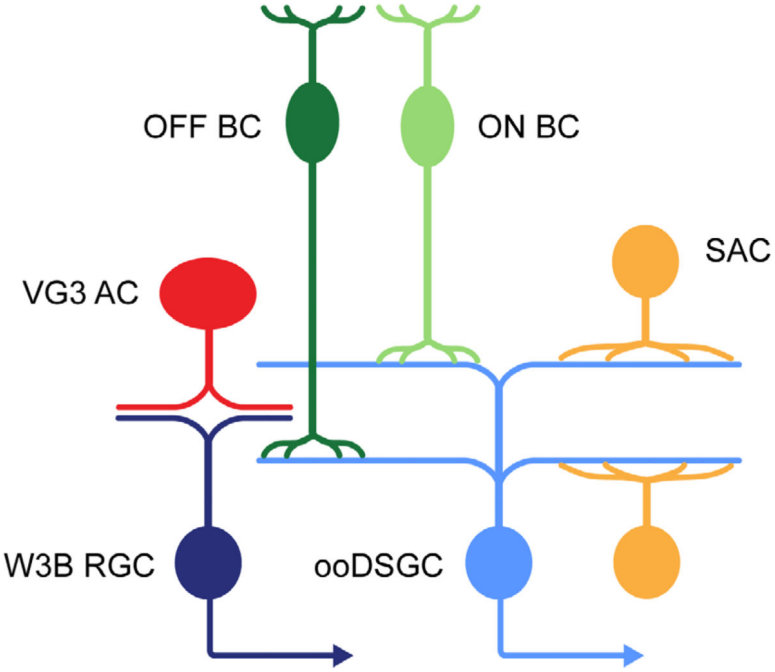

FIGURE 5.

Diagram of neuron types in the inner retina. OFF bipolar cells (BCs) (light green), ON BCs (dark green), starburst amacrine cells (SACs) (orange), VG3 amacrine cells (VG3 ACs)(red), W3B retinal ganglion cells (W3B RGCs) (dark blue), and ON/OFF direction-selective ganglion cells (ooDSGCs) (light blue)

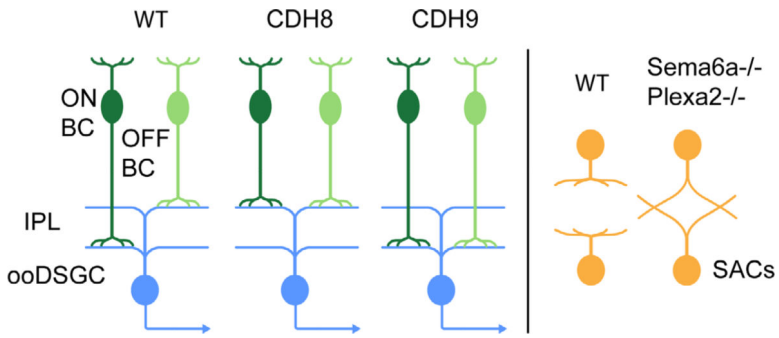

FIGURE 6.

Laminar targeting in the IPL. Wildtype retinal ganglion cells (RGCs), bipolar cells (BCs), and starburst amacrine cells (SACs) are depicted on the left of each panel. Duan et al. found that a subtype of OFF BC selectively expresses CDH8, whereas a subtype of ON BC selectively expresses CDH9. They showed that ectopic expression of either of these molecules is sufficient to instruct the divergent laminar targeting (left). Sun et al. showed that loss of SEMA6A-PLEXA2 results in loss of segregation between ON and OFF SACs (right)

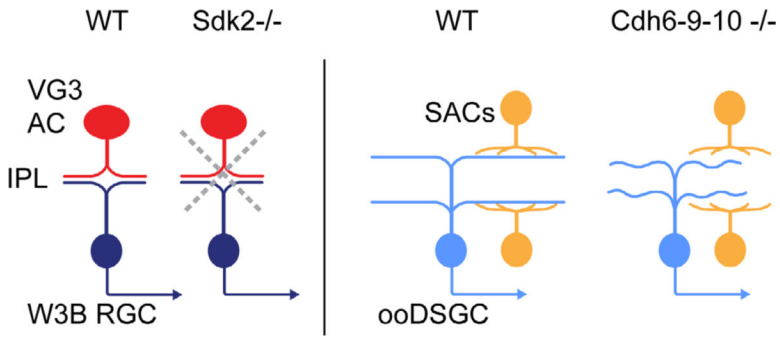

FIGURE 7.

Synapse formation in the IPL. Wildtype retinal ganglion cells (RGCs) and amacrine cells (ACs) are depicted on the left of each panel. Krishnaswamy et al. found that SDK-2 is localized at the synapses between VG3 ACs and W3B RGCs, and that loss of SDK-2 prevents proper synapse formation between these two neurons (left). Duan et al. found that only the combined loss, but not the loss of one or two, of CDH6, CDH9, and CDH10 results in loss of tight cofasciculation between ON/OFF direction-selective ganglion cells (ooDSGCs) and starburst ACs (SACs) (right)