Photoreceptor Fate Determination in the Vertebrate Retina

Special Issue

Photoreceptor Fate Determination in the Vertebrate Retina

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Photoreceptors are highly specialized primary sensory neurons that sense light and initiate vision. This critical role is well demonstrated by the fact that visual impairment accompanies photoreceptor loss or dysfunction in many human diseases. With the remarkable advances in stem cell research, one therapeutic approach is to use stem cells to generate photoreceptors and then engraft them into diseased eyes. Knowledge of the molecular mechanisms that control photoreceptor genesis during normal development can greatly aid in the production of photoreceptor cells for this approach. This article will discuss advances in our understanding of the molecular mechanisms that regulate photoreceptor fate determination during development. Recent lineage studies have shown that there are distinct retinal progenitor cells (RPCs) that produce specific combinations of daughter cell types, including photoreceptors and other types of retinal cells. Gene regulatory networks, in which transcription factors interact via *cis*-regulatory DNA elements, have been discovered that operate within distinct RPCs, and/or newly postmitotic cells, to direct the choice of photoreceptor fate.

Keywords: photoreceptor fate determination, retinal development, gene regulatory network, transcription factors, cell lineages

The vertebrate retina is a highly evolved organ that captures and processes visual information within the eye, and delivers the resultant signals to the brain.[1] More than 60 retinal cell types interact within circuits that transform the information conveyed by light, processing it to extract features of relevance to an organism, and then delivering that information to the brain.[2] Among these, photoreceptor cells carry out phototransduction to initiate the process of vision.[3] Unfortunately, photoreceptors are relatively vulnerable to environmental perturbations and genetic insults, possibly due to their high metabolic activity and delicate structure.[4] In many human retinal diseases, such as retinitis pigmentosa and macular degeneration, visual impairment is due to photoreceptor dysfunction, typically followed by degeneration.[5,6] Therapies aimed at improving photoreceptor survival and/or function have the potential to significantly slow down the loss of vision, or even improve vision. From both a basic science and a clinical perspective, photoreceptors have been the subject of many studies, and significant progress has been made regarding their development and function. This article will focus on the molecular mechanisms that control photoreceptor cell fate determination during development, with the hope of informing stem cell-based photoreceptor generation in vitro for cell replacement therapies.

During development, rods and cones are produced in a conserved temporal order, along with other cell types in the retina.[7] In most species, cones are born before rods, but there can be overlap in their birthdates. In mice, the production of cones starts at around embryonic day 10 (E10), peaks at E14, and finishes before birth at E18. Rods are born over a longer period of time during development, starting at around E13, reaching a peak at birth, and continuing until postnatal day 7 (P7).[8–10] At different developmental stages, different numbers of rods and cones are generated. The number of newborn cells fated to be rods exceeds that of cones quickly after the start of rod genesis; and by E14, there are more cells fated to be rods than there are cells fated to be cones.[8] Upon completion of development, rods comprise approximately 70% of all cells in the mature retina, while cones comprise approximately 2%.[9] These studies lay the groundwork for understanding rod and cone fate determination, and suggest that different molecular mechanisms may control photoreceptor fate decisions at distinct developmental stages. One aspect of the mechanisms used is the role played by retinal progenitor cells (RPCs), the mitotic cells that produce retinal cells. These cells, and/or their newly postmitotic progeny, are the cells in which cell fate decisions are made.[11] It is thus important to consider the nature of RPCs at different stages, as well as the gene regulatory networks (GRNs) that operate within them and/or their newly postmitotic progeny at different developmental stages. Recent advances that define these aspects of photoreceptor determination will be presented here.

Distinct RPCs Produce Rod or Cone Photoreceptors

All retinal cells, including photoreceptors, are derived from RPCs.[12] A longstanding question in this field is whether RPCs differ in terms of their ability to produce specific types of retinal cells, as has been discussed in a recent review.[11] Lineage studies have shown that the descendants of single RPCs, that is, clones, often comprise many retinal cell types, indicating that RPCs can be multipotent. In the mouse and rat, which have a high proportion of photoreceptors, almost every clone has a rod, and clones initiated early also have cones. While some clones include only photoreceptors, others include photoreceptors along with other retinal cell types. This finding may

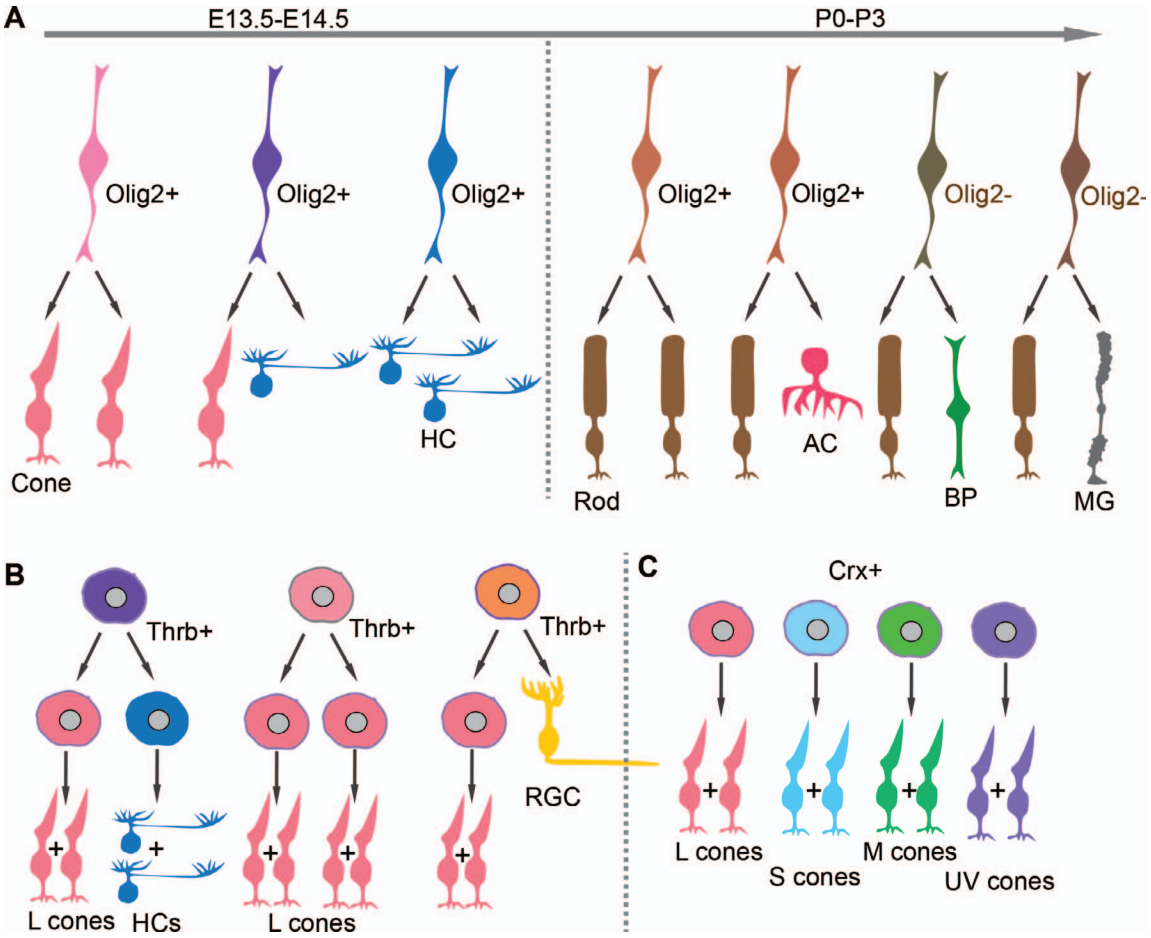

Figure 1. Distinct RPCs produce specific retinal cell types. (**A**) Retroviral lineage tracing was directed to RPCs that express the bHLH TF, *Olig2*, at embryonic and postnatal stages in the mouse retina.[14] Almost all resulting clones were only 1 or 2 cells, revealing that *Olig2*-expressing RPCs were terminally dividing. *Olig2*-expressing RPCs infected at E13.5 to E14.5 produced almost exclusively cones and horizontal cells. RPCs marked by a retrovirus that did not specifically target *Olig2*-expressing RPCs produced larger clones (average size = 32 cells), some of which included retinal ganglion cells (RGCs) from infection at this time. *Olig2*-expressing RPCs infected at P0 or P3 produced almost exclusively rods and amacrine cells. RPCs infected by a retrovirus that did not specifically target *Olig2*-expressing RPCs produced rods, bipolar cells (BP), and Müller glia (MG).[53] (**B**, **C**) Homotypic pairs of cones are made by RPCs in zebrafish.[15] (**B**) Live imaging of zebrafish RPCs expressing a reporter for *Thrb* showed that they produce long-wavelength cones (L cones), horizontal cells (HCs), and retinal RGCs. The L cones were made in terminal divisions. (**C**) RPCs expressing a reporter for *Crx* were terminally dividing and produced homotypic pairs of cones that expressed the long (L), medium (M), short (S), or UV opsin.

indicate that some RPCs are committed to produce only photoreceptors whereas other RPCs are multipotent. Recent studies have probed this question by using molecular markers that distinguish among RPCs, and then tracking the type of progeny that such RPCs make. The results from these studies provide strong evidence for intrinsic differences between those RPCs that produce rods and those that produce cones.

Single-cell expression profiling of RPCs has been carried out using microarrays to probe whether RPCs differ from each other.[13] These data showed many differences among RPCs across development, as well as at a single time in development. One gene that varied was the bHLH gene, *Olig2*, which showed variation in expression among RPCs across time, and at one time. Hafler et al.[14] followed the cell types produced by *Olig2*-expressing RPCs, and showed that they were terminally dividing and produced specific pairs of neurons at different developmental stages in mice. When the daughters of E13.5 to E14.5 *Olig2*-expressing RPCs were clonally labeled by retroviral infection, only cones and horizontal cells were marked. When day P0 or P3 *Olig2*-expressing RPCs were marked by viral infection, only rods and amacrine cells were labeled. The *Olig2*-minus RPCs made clones comprising rods and bipolar cells, as well as rods and Müller glial cells (Fig. 1A). Interestingly, though *Olig2*-expressing RPCs clearly can make both rods and cones, the *Olig2*-derived clones never comprised both rods and cones, even at E13.5 to E14.5, when the birthdates for rods and cones overlap.

A study in zebrafish tracked the progeny of RPCs that expressed a reporter for a cone marker, *Thrb* (also known as Nr1a2), by live imaging.[15] These *Thrb*-expressing RPCs produced predominantly long (L) cones in terminal divisions. In addition to these terminal divisions, a few *Thrb*-expressing RPCs produced 4-cell clones. One such clone comprised 2 L cones and 2 horizontal cells, each made by symmetrical terminal divisions. Additional types of divisions were observed as well, but no clones of rods and cones were observed (Fig. 1B). The authors also examined the *Crx*-expressing RPCs by using a reporter based upon *Crx*, which is expressed in RPCs, rods, cones, and bipolar cells.[16] *Crx*-expressing RPCs also showed homotypic patterns from terminal divisions, producing pairs of cones expressing the same opsin type (i.e., the

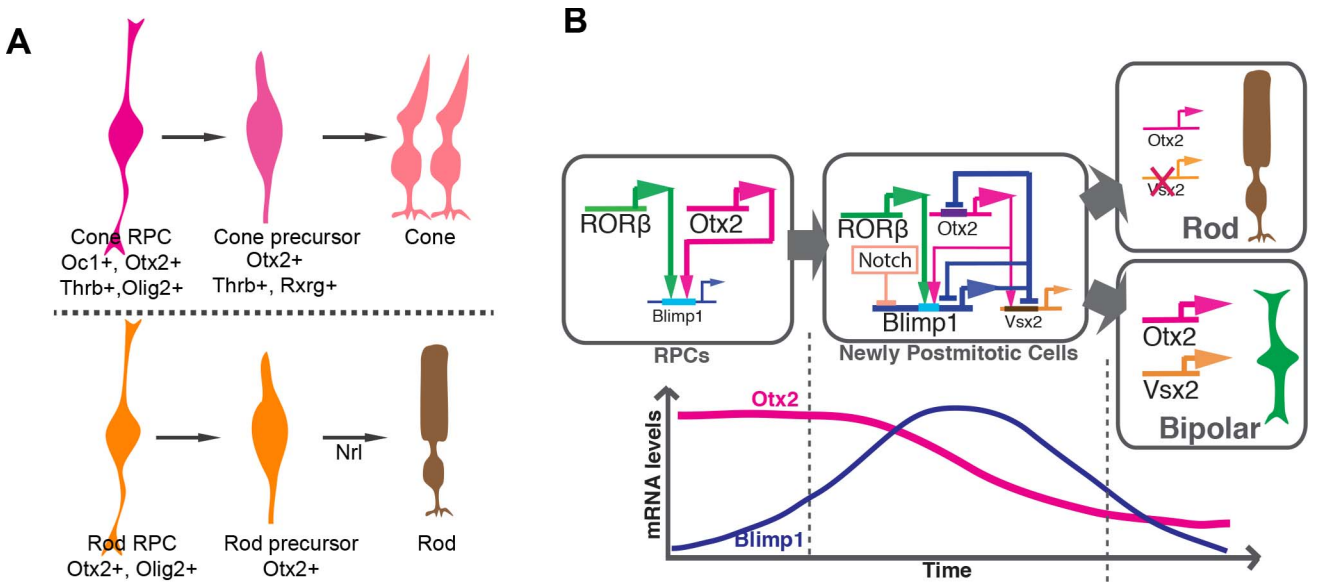

Figure 2. Models for photoreceptor fate determination. (**A**) A model for rod versus cone development wherein distinct RPCs produce cones and rods.[14,44] RPCs that express *Olig2*, *Otx2*, and *Oc1* are present in the early retina. Both *Oc1* and *Otx2* are required for expression of the early cone marker, *Thrb*, and to produce cones. These early RPCs also can produce horizontal cells, which upregulate *Oc1*, while cones downregulate *Oc1*. Rods are produced by RPCs that express *Olig2* and *Otx2*, but not *Oc1*. The newly postmitotic cells are modeled to be distinct from the point of genesis from those made by the *Oc1*-expressing RPC. Newly postmitotic cells made by any of these RPCs are likely to require additional steps to determine their fates; for example, they need to escape Notch signaling and set the proper level of *Otx2*. Additional genes expressed by the RPCs and/or newly postmitotic cells that are also important in induction, or repression, of the rod and cone fate are *Rax*, *Pax6*, *Blimp1*, *RORβ*, *Vsx2*, and multiple bHLH genes. (**B**) GRN that regulates the binary fate choice of rod versus bipolar cell. As cells exit mitosis, *Otx2* and *RORβ* are expressed and induce expression of *Blimp1* through the B108 enhancer.[39] During and after cell cycle exit, *Blimp1* levels rise, whereupon *Blimp1* negatively regulates the expression of *Otx2* through the ECR2 enhancer[50] as well as its own expression, through a *Blimp1* 3′ UTR element.[39] *Blimp1* also negatively regulates *Vsx2* through at least two enhancers.[37,54,55] *Otx2* primes expression of *Vsx2*[38,55] and Notch represses, directly or indirectly, the level of *Blimp1*.[20,56] Cells that have low *Otx2* and no (or low) expression of *Vsx2* achieve the rod fate, whereas those with high *Otx2* and *Vsx2* achieve the bipolar fate. The mRNA levels of *Otx2* and *Blimp1* are dynamic throughout this period via feedforward and feedback regulation. Part (**B**) reprinted with permission from Wang S, Sengel C, Emerson MM, Cepko CL. A gene regulatory network controls the binary fate decision of rod and bipolar cells in the vertebrate retina. *Dev Cell*. 2014;30:513–527. Copyright 2014 Elsevier, Inc.

medium [M], short [S], long [L], or ultraviolet [UV] cone opsins) (Fig. 1C).

Both of these studies suggest that there are distinct types of terminally dividing RPCs that produce rods or cones. Further examination of the heterogeneity of RPCs and characterization of their lineage history will be needed to fully understand the lineage trees that result in these terminally dividing cells.

GRNs Involved in Cone or Rod Genesis

The lineage experiments described above suggest that the decision to be a photoreceptor, that is, the determination event, occurs in terminally dividing cells and/or their newly postmitotic progeny, and may occur over a period of several days. Many genes have been shown to be involved in photoreceptor fate determination, including *Notch1*,[17–22] *Rax*,[23–26] *Otx2*,[27–29] bHLH genes,[30–35] *Blimp1*,[36–39] *Vsx2*,[40–42] *Foxn4*,[43] and *Oc1*.[44] Loss of function of these genes leads to a reduction in the number of photoreceptors, with a concomitant increase in one or more other cell types. Given that multiple genes are involved in the fate determination events, it is likely that GRNs are at work in the retina to effect the timely production of the correct number of each type of photoreceptor. Recent studies have started to dissect how transcription factors (TFs) interact within GRNs in distinct RPCs to control rod and cone photoreceptor fate determination.

As in zebrafish, *Thrb* is an early marker of cones in mice and chicks.[45] Through discovery of an enhancer that regulates *Thrb* in these species, along with the cognate TFs that regulate the enhancer, a TF that is important for cone determination, Onecut 1 (*Oc1*), was discovered.[44] An understanding of the role of *Oc1* has aided in the definition of a GRN for cones versus rods. *Otx2*, which was previously shown to be important for rod and cone genesis,[27] and *Oc1* were shown to combinatorially regulate the *Thrb* gene via direct binding to the ThrbCRM1 enhancer, which is active in an RPC that generates horizontal cells and photoreceptors. *Oc1* was found to be expressed in chick and mouse RPCs during the period when cones are generated, but not in the postnatal mouse retina, when only rods, and not cones, are produced (Fig. 2A). Misexpression of *Oc1* in the postnatal mouse retina, where *Otx2* is expressed, induced the formation of immature cones, along with horizontal cells. This induction was dependent upon *Otx2*, as removal of a conditional allele of *Otx2* prevented this induction. These data suggest that *Otx2* and *Oc1* together promote the fates of cones and horizontal cells. A model for *Oc1* and *Otx2* action in the retina was also proposed.[44] The CRM1-active RPCs divide to give rise to cones and horizontal cells. In cone precursor cells, the level of *Oc1* declines and *Otx2* is maintained, while in horizontal cell precursors, the level of *Oc1* increases and *Otx2* decreases. More interestingly, *Oc1* probably plays an important role in cone versus rod fate determination, as the repression of *Oc1* led to increased rod genesis. Specifically, electroporation of the chick retina with a construct in which a transcriptional repressor domain was fused to *Oc1* led to a reduction in *Thrb* expression. This construct also led to an upregulation of *MafA*,

the chick homologue of *Nrl*,[46] a key gene in rod differentiation.[47] The *Oc1*-repressor domain fusion also led to premature expression of rhodopsin, in keeping with an increase in the production of rods. Moreover, in *Oc1* knockout mice, a reduction in *Thrb* mRNA and an upregulation in *Nrl* mRNA were seen. These data all point to a role of the *Oc1* gene (and possibly *Oc2*, which has high homology to *Oc1*) in regulating the cone versus rod fate decision. In summary, this study indicates that coexpression of *Otx2* and *Oc1* may be able to drive early events in cone genesis, leading to cone induction from stem cells.

A GRN that controls the binary fate decision between rod photoreceptor and bipolar cells in postnatal RPCs has also been recently discovered.[39] At postnatal stages in the mouse retina, several TF genes were known to regulate the rod versus bipolar fate choice, including *Notch1*,[17,20] *Otx2*,[28] *RORβ*,[48] *Vsx2* (*Chx10*),[40] and *Blimp1*.[36,37] *Otx2* had been strongly implicated to be a direct regulator of *Vsx2*[38,49] and *Vsx2* to be a direct target of *Blimp1*.[37] As *Blimp1* could be considered as a node in this GRN, the enhancer(s) that regulates *Blimp1* in the postnatal mouse retina was of interest.[39] An enhancer for *Blimp1* of only 108 base pairs (B108) was identified. The new method of Cas9-genome editing was used to delete B108 from the mouse genome in vivo using electroporation. B108 deletion recapitulated the retinal loss of function phenotype for *Blimp1*, thereby establishing that B108 is required for *Blimp1* activity in the retina. Feedforward and feedback interactions were then worked out using electroporation of enhancer constructs for *Otx2*[50] and *Blimp1*, as well as gain and loss of function experiments for other TFs of this GRN. Quantification of mRNA levels for some of the genes in the network was accomplished using the single-molecule fluorescent in situ hybridization method of Raj and van Oudenaarden.[51] These studies revealed that the critical output of this GRN is the level of expression of *Otx2* and *Vsx2* (Fig. 2B). High *Otx2* and no (or low) *Vsx2* are required for the rod fate, whereas high *Otx2* and *Vsx2* are required for the bipolar fate.

These studies provide examples of GRNs that drive photoreceptor fate determination and highlight the complexity of such networks, showing both feedforward and feedback loops of regulation. They also underscore the complexity of interactions that will need to be teased apart for an understanding of cell fate decisions in complex tissues, as well as the need to use quantitative assays for gene expression levels, given that the levels of these TFs are critical in directing the fate choice.

Summary and Future Issues

Work over the last several years has greatly contributed to our understanding of the fate determination of rods and cones. Rather than rods and cones being produced as a generic type of photoreceptor, that later chooses to be a rod or a cone, it appears that each type of photoreceptor is produced as a rod or a cone by its RPC. Distinct types of RPCs that are terminally dividing produce the different types of cones as homotypic pairs. There are likely many types of RPCs with different GRNs in operation that produce rods, in combination with different types of siblings in terminal divisions; for example, *Olig2*-expressing RPCs can produce a rod and an amacrine cell while *Olig2*-negative RPCs can produce a rod and a bipolar cell.

These recent data raise several questions for future research as well as providing possibilities for stem cell therapies. First, the GRNs that dictate the formation of rods versus cones, and of different cone types, will need to be characterized. The determination events will need to be linked to the regulatory events, for example, chromatin configuration and microRNAs (e.g., see Busskamp et al.[52]) that direct and/or maintain specific gene expression in differentiating cells. Second, the heterogeneity of RPCs needs to be further explored. The RPCs that are upstream of the terminally dividing RPCs that produce different types of daughter cells will need to be defined to determine if there are distinct lineages that include more than the terminally dividing RPCs. Third, the GRNs that control photoreceptor maturation and function will need to be uncovered to understand how these GRNs are dysregulated in retinal diseases.

By addressing these issues, we would gain a more comprehensive understanding of photoreceptor development, which can lead to novel strategies for the efficient generation of photoreceptor cells or precursors with better transplantation potential from stem cells. For example, it might be possible to label and enrich for distinct RPCs that are biased to produce cones or rods during directed differentiation of stem cells. We may also be able to monitor and manipulate the in vitro differentiation process in a stage- and cell type-specific manner by utilizing *cis*-regulatory elements that integrate regulatory information within GRNs.

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