Photoreceptor subtype specification: from flies to humans

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Multiple cell types often differentiate from a pluripotent cell. These cells may then further diversify as distinct subtypes. The visual system provides an ideal model for studying subtype specification as various photoreceptors acquire different functions based on the type of opsin they express. Opsin expression is mostly controlled through transcriptional mechanisms that are evolutionary conserved from Drosophila to humans. In addition, it appears that, from a ‘default’ developmental state, distinct ‘acquired’ photoreceptor states develop upon receiving intrinsic or extrinsic signals. This review discusses factors involved in opsin gene regulation and how their integration may explain how subtype specificity is achieved.

Key words: LCR / opsin / Otd / photoreceptor / transcription factors

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Dual system for vertebrate vision: cones versus rods

Vision results from the reception of light by photoreceptor cells and its transduction and processing into a neural signal in the retina. This signal is then transmitted to the brain where it is integrated into an image (for an excellent overview of vertebrate vision, visit http://webvision.umm.edu/webvision/). In vertebrates, two systems have evolved to perform different visual tasks [Figure 1(a)]. Image formation in dim light primarily involves the rods, highly sensitive photoreceptors that respond to a broad spectrum of wavelengths, but are easily bleached in intense light. In bright light, most of the visual functions are achieved through the fovea, a bi-functional region at the center of the retina that is composed essentially of cone cells. The dense packing of cones in this region allows high-resolution image formation.

Cone cells can be divided into distinct groups based upon which opsin molecule they express. Opsins are the light-collecting visual pigments that are densely arranged in the apical outer membrane of photoreceptors. When excited by a photon, vertebrate opsins initiate a G-protein-coupled signal transduction pathway that leads to the excitation of bipolar cells, while ganglion cells transmit the signal to the brain. To discriminate between different wavelengths of light, the opsin gene family has expanded through a series of gene duplications to form a group of proteins with unique light response properties.

In order to avoid sensory overlap, most sensory systems have adopted the general rule of ‘one receptor cell/one receptor molecule’, and indeed, with rare exception, a given photoreceptor expresses a single opsin gene. Vertebrate rods, for example, all express rhodopsin, the rod opsin molecule that absorbs wavelengths within a broad range centered around 500 nm. Cone cells, on the other hand, express one of several cone opsins. Humans, for example, have three cone cell populations, S, M, and L, whose opsins absorb short (<500 nm, blue), medium (~530 nm, green), and long (~560 nm, red) wavelengths, respectively, to give rise to our trichromatic visual system. Other organisms, however, use different sets of opsins to achieve optimal visual perception in their specific environment (see Ahnelt and Kolb for an excellent review). Most mammals, for instance, display a dichromatic system with only S and M cones, while...
Figure 1. Photoreceptor subtypes in humans (a) and flies (b) and (c). (a) M (green), and L (red) cones are concentrated in the center of the retina and surrounded by S (blue) cones in the fovea to give rise to high resolution image formation as well as color perception. Rod cells, important for dim light vision, are present primarily at the outer perimeter of the retina and all express the rod opsin, rhodopsin. Photoreceptors lie in the outer layer of the retina, so light must pass through the processing neurons (e.g. bipolar, amacrine, horizontal, and ganglion cells) before reaching the photoreceptors. (b) The six outer photoreceptors in the ommatidia of \textit{Drosophila} contain a single rhodopsin, Rh1, whereas the inner photoreceptors express a complex pattern of \textit{rhs} that divide the ommatidia into two subtypes, \textit{pale} (p) and \textit{yellow} (y). In addition to the difference in their \textit{rhodopsin} genes, the inner and outer photoreceptors also differ in the size of their rhabdomeres and the region of the optic lobe where their axons project. (c) Visualization of the random distribution of \textit{p} versus \textit{y} ommatidia in the adult \textit{Drosophila} eye as detected by antibody staining against the Rh5 (blue) or Rh6 (green) rhodopsins (kindly provided by Remi Sonneville).

animals such as birds or fish can express four or more different cone opsins together with filtering pigments, such as oil droplets. Thus, photoreceptors comprise a heterogeneous population of specialized cells that together are capable of detecting a broad range of spectral sensitivities and visual clues.

In addition to the number of visual pigments that each organism expresses, the distribution and relative abundance of each photoreceptor cell population varies to reflect specific needs in the visual field. In humans, rods comprise \(\sim 90\%\) of photoreceptors and are mostly present at the periphery of the retina, whereas the cones are concentrated in the fovea.\(^5,6\) Largely nocturnal animals such as mice, however, rely primarily on rod-based vision, have even fewer cone cells (\(\sim 5\%\) of all photoreceptors) and lack a fovea.\(^7\) Nevertheless, their rods and cones maintain an organized distribution: S cells form a dorsal-to-ventral density gradient through the retina while M cells, although stochastically distributed, express higher levels of their green-like opsin in the dorsal region of the eye.\(^8,9\) Interestingly, mice also have a relatively large subset of cones that coexpress blue and green opsins, a result that contradicts the ‘one receptor cell/one receptor molecule’ rule.\(^8,10\)

Although the function of these cells is unclear, it likely that this occurs due to the fact that these nocturnal animals do not rely on color vision. Nevertheless, their distribution is found in a defined pattern in the retina. Based on the defined ratio of cone to rod cells as well as the spatial distribution of specific subtypes of photoreceptors, it is clear that opsin genes are under extensive and tightly-controlled regulation.

**Cell-specific opsin gene expression in \textit{Drosophila}**

Despite its very different morphological characteristics from the single lens eye of vertebrates, the compound eye of \textit{Drosophila} shares similar mechanisms for achieving visual perception. The adult \textit{Drosophila} eye is composed of \(\sim 750\) independent ‘eyes’, or ommatidia, each organized in a precise hexagonal array. Every ommatidium contains eight photoreceptors: six outer photoreceptors (R1–R6) and two inner photoreceptors (R7 and R8) and each expresses one of five different \textit{rhodopsin} genes (\textit{rhs}) [Figure 1(b)]. As in vertebrates, there are
two visual systems that specialize in different tasks.\textsuperscript{2} The outer photoreceptors have large rhabdomeres (the structures that gather light) and contain Rh1, a green-centered Rh with a broad spectrum of absorption. They are involved in image formation and motion detection and function well in dim light.\textsuperscript{11} They resemble the vertebrate rods in that they express a broad-spectrum opsin and represent the most sensitive photoreceptors. However, like cones in the fovea, they also support relatively high-resolution image formation. In contrast, the inner photoreceptors appear to be mostly involved in color discrimination and, thus, resemble this specific subfunction of vertebrate cones. They have smaller rhabdomeres than outer photoreceptors and express a much more complex pattern of \textit{rh} genes. This expression pattern divides the ommatidia into two distinct subtypes: pale (p) and yellow (y) (Figure 1(b)). p ommatidia express \textit{rh3} in R7 cells and \textit{rh5} in R8 cells, while y ommatidia have \textit{rh4} in R7 and \textit{rh6} in R8\textsuperscript{12} (also Tahayato \textit{et al.}, in preparation). These two subtypes of ommatidia are stochastically distributed throughout the eye but are present in a very defined ratio, with 30\% of the ommatidia being of the p subtype and the remaining 70\% being of the y subtype. It is likely that the presence and distribution of these two subclasses allow distinction of a broader range of wavelengths and present the inner photoreceptors as a good system to achieve color vision. In addition to their differences in morphology and opsin expression, outer and inner photoreceptors differ in where their axons project: outer photoreceptors project to the lamina part of the optic lobe, while inner photoreceptors project deeper to the medulla where inputs are compared to achieve color discrimination.\textsuperscript{11} Therefore, tightly controlled molecular pathways must exist to not only restrict the expression of a given opsin to a subtype of photoreceptors, but to also specify where these subsect connect in the brain.

**Transcriptional control of \textit{rh} expression**

Several lines of evidence suggest that the distribution of opsins is regulated purely at the transcriptional level.\textsuperscript{13–15} Photoreceptor subtype-specific expression of the different \textit{Drosophila} \textit{rh} genes, for instance, is regulated by promoter sequences of less than 600 bp.\textsuperscript{13,14,16} Phylogenetic as well as molecular genetic studies have revealed that such minimal \textit{rh} promoters have a modular and relatively simple bipartite organization: a common proximal element confers photoreceptor identity (RCSI, rhodopsin conserved sequence I), while upstream \textit{rh}-specific sequences provide subtype specificity (RUS, rhodopsin upstream sequences)\textsuperscript{16} (Figure 2). These early studies also demonstrated that the RCSI sequences were relatively interchangeable among promoters, while the RUS sequences could independently drive subtype-specific gene expression in chimeric promoters.

Our laboratory has recently shown that the transcription factor Pax-6 acts through the RCSI sequence.\textsuperscript{14,17} Pax-6 was the first gene to be shown to induce the differentiation of eye structures when ectopically expressed in different regions of \textit{Drosophila}.\textsuperscript{18} Furthermore, mutation of this gene in flies, mice, and humans lead to the lack of eye structures (see Gehring and Ikeo,\textsuperscript{19} also reviews by Kumar and Moses, and by Hanson in this issue). Thus, Pax6 has been termed a ‘master regulatory gene’ for eye development. Several studies have since implicated Pax-6 in later roles of eye development as well.\textsuperscript{20} A role for Pax6 in \textit{rh} gene expression was suggested by the observation that the RCSI site represents a palindromic binding site for a dimer of paired-class homeodomain transcription factors, a class of proteins that includes Pax6.\textsuperscript{17} Biochemical and genetic studies demonstrated that Pax6 does bind the RCSI through its homeodomain and is
responsible for the regulation of each of the rh genes. Recent work by Papatsenko et al.\textsuperscript{14} also showed that although every rh promoter contains a RCSI site, this site varies in consistent ways from one gene to another and appears to contribute to photoreceptor subtype-specificity. For instance, replacement of the RCSI from the pR7 rh3 promoter with that of rh5 or rh6 leads to de-repression of rh3 expression to all R7 cells, suggesting the presence of a yR7 repressor element within the rh3 RCSI site. Interestingly, further examination of individual RCSI sites reveals, at least in some cases, the presence of binding sites for additional transcription factors, including the homeodomain protein, orthodenticle (Otd). Together, these data suggest that Pax6 interacts directly with other factors to direct subtype-specific rh gene expression.

Pax6 is not the only factor that regulates several rh genes. For instance, Otd and its vertebrate homologues appear also to have a conserved role in controlling opsin expression in mammals and in flies. Otd is a member of a family of proteins that plays an evolutionarily conserved role in the formation of anterior part of the brain, from the lowest animal forms to humans.\textsuperscript{21} The Otd family, comprised of Drosophila Otd and vertebrate Otx-1, Otx-2 and Crx, are homeodomain transcription factors distinguished by the presence of a lysine (K) at residue 50 of their homeodomain. This residue is critical for determining DNA-binding specificity of the homeodomain.\textsuperscript{22} In most homeodomains, residue 50 is a Q and confers binding to the sequence TAAT\textsuperscript{512}. In Otd, the K leads to the recognition of the sequence TAATCC. Interestingly, this sequence is present in several photoreceptor-specific promoters, including Drosophila rh3, rh5, and rh6, as well as vertebrate rod opsin, blue and green cone opsins, and interphotoreceptor retinoid-binding protein (IRBP).\textsuperscript{23} Indeed, Crx has been shown to regulate opsin genes in the vertebrate retina, while mutations in Crx lead to cone–rod dystrophy and deletion of Crx-binding sites has been associated with blue cone monochromacy.\textsuperscript{24} Recent data from our laboratory have also revealed a role for otd in the positive regulation of p-type rh5 in inner photoreceptors and in the negative regulation of y-specific rh6 in outer photoreceptors (Tayahato et al., in preparation). While in flies this subtype-specific difference in Otd function is not yet understood, increasing evidence suggests that other proteins interact with Crx to control gene expression in mice.\textsuperscript{25–27} Mitton et al.,\textsuperscript{25} for instance, have shown that the photoreceptor-specific transcription factor NRL interacts with Crx to synergistically activate rod opsin gene expression in cultured mammalian cells. In addition, the data described earlier strongly suggest that Otd associates with Pax6 in flies. Therefore, although Otd is expressed in all photoreceptors, it may associate with other subtype-specific transcription factors in order to function as an activator in pR7/R8 cells and as a repressor in outer photoreceptors.

As in flies, vertebrate photoreceptor specificity is achieved by short promoter regions that consist of several important regulatory sites, including Ret-1/PCE-1, BAT-1, Ret-4, and NRE boxes (see Chen et al.\textsuperscript{27} and references therein). These elements are not only present in the opsin promoters, but also appear to participate in photoreceptor-specific expression of a broad range of phototransduction-associated genes, including arrestin and IRBP. Thus, transcriptional complexes bound to these sites may provide a general regulatory mechanism for establishing the co-expression of functionally linked proteins. Many of the factors involved in this regulation have been identified and include Rx, Crx, and NRL.\textsuperscript{26–29} Interestingly, all three of these proteins also participate in earlier events of photoreceptor development.\textsuperscript{30–32} As opsin expression is a very late event in photoreceptor differentiation, this finding suggests that early eye determination has taken advantage of pre-existing genetic pathways used for photoreceptor differentiation. The completion of genome projects might allow the identification of other players involved in photoreceptor determination/differentiation by searching for genes containing this type of precisely organized regulatory regions. Despite this advance in understanding general photoreceptor expression, however, the factors responsible for achieving expression in different subsets of photoreceptors in later development remain largely unknown.

A mechanism of opsin exclusion: the LCR

It is clear that exclusion of expression among different opsin genes is almost absolute in both flies and humans, and is controlled essentially at the transcriptional level. Exclusive expression of a single opsin gene per photoreceptor is an important feature for avoiding sensory overlap and allowing color perception. Other sensory organs require similar exclusivity, underscoring the importance of identifying the mechanisms involved in the mutual
exclusion of gene expression. However, little is known regarding these control mechanisms.

One solution for achieving exclusive expression among multiple gene products that has arisen in Nature is the use of a locus control region (LCR). 35 The concept of an LCR was originally developed in the hematopoietic system where it controls the temporal expression pattern of the β-like globin genes throughout development. The β-globin LCR is a complex element located ~20 kb upstream of a clustered array of five globin genes which are turned on and then off in a linear, 5′-to-3′ fashion during successive stages of development. Since its original description, a number of LCRs have been identified that are important for achieving proper expression of their associated gene(s) in transgenic animals. Although the way in which LCRs function is not precisely known, most models involve interactions between proximal promoter-specific factors and LCR-associated proteins. Furthermore, they suggest that the LCR can only contact one proximal promoter at a time, thus ensuring a ‘clean’ switch from one gene to another.

Most opsin genes are present at distinct chromosomal loci, and thus are unlikely to utilize an LCR-mediated form of regulation. One exception, however, is the red (L)/green (M) opsin gene cluster in humans. These genes are juxtaposed on the X chromosome and share over 98% identity. As M (but not L) opsin is present in all other mammals, it is likely that L opsin arose from a recent gene duplication of M opsin in Old World primates. 34 The presence of such highly related genes near one another has also led to further amplification of the M/L cluster. However, only two to three of these genes are generally expressed, with the 5′ genes in the cluster expressed more often than the 3′ genes (see Nathans 33 and references therein), suggesting that a higher level of regulation is involved. Indeed, proper L and M opsin gene expression not only requires their individual proximal promoters, as in flies or other vertebrates, but also requires an LCR element. 35 The LCR is located ~3.5 kb upstream of the cluster, with the L opsin gene being 5′ to the M opsin(s). Similarly to the globin genes, LCR and promoter-specific factors interact to activate transcription and allow the mutual exclusion of the L and M opsins within cone cells.

Wang et al. 35 have recently proposed two models for how the opsin LCR participates in the subtype-specific expression of the L and M opsins: a ‘standard model’ in which cone subtype-specific factors bind to the individual promoters and become activated by interaction with a common LCR that acts as a simple enhancer; or a ‘stochastic model’ in which both promoters bind the same M/L cone factors but only one can stochastically associate with the LCR in individual cells to allow expression of a single gene (Figure 4). Because this model relies on a random decision between L versus M promoters, the two alleles could make different choices, leading to opsin co-expression. The fact that the L/M cluster is located on the X chromosome circumvents this problem, however, as only one X is functional in any given cell.

To test the two models in vivo, Wang et al. 35 transferred an artificial human LCR L/M gene array into mice. Because mice have M, but not L, cone opsin, the hypothesis was that if the choice between L versus M opsin was based on cell-type specific factors (i.e. the ‘standard’ model), mice should be unable to express the human L gene because they would lack the necessary factors. In contrast, in the ‘stochastic’ model, both genes should be expressed alternatively. The L promoter was expressed in 63% of cone cells, the M promoter in 10%, and both promoters were co-expressed in the remaining 27%. These findings suggest that similar factors are associated with each opsin promoter and, although full exclusion was not achieved, support the stochastic model. It should be noted that mice normally co-express S and M opsins in a subset of photoreceptors, suggesting that this nocturnal animal has lost the necessary factors important for mutual exclusion between S and L/M opsins found in other species.

Although the factors involved in this regulation are not precisely known, the mutation of Crx-related binding sites within the LCR of humans leads to blue (S) cone monochromacy; 23 suggesting that this ubiquitous photoreceptor-specific factor is involved in LCR-mediated regulation. In addition, Crx is required in both mice and humans for cone opsin expression; 36 further supporting the finding by Wang et al. that similar factors could be used in both systems. Continued work on the LCR element should be useful for identifying additional factors involved in L and M opsin regulation.

A default pathway for photoreceptor development?

Whilst the mechanisms of action for establishing photoreceptor subtypes is not understood, it is clear
that once the choice of an opsin gene is made, other cells must be notified of this choice. These include the target neurons that must interpret the correct signal, as well as other photoreceptors that must achieve coordinated and mutually exclusive rhodopsin expression. For instance, in Drosophila, rh expression is coupled between R7 and R8 photoreceptors: rh4 and rh6 are always coordinately expressed in 70% of R7 and R8 cells, respectively, whereas the rh3/rh5 pair is present in the remaining 30% of R7/R8.12 Interestingly, in the absence of R7 cells, all R8 cells express rh6. In contrast, removal of R8 cells prior to rh expression has no effect on the percentage of rh3 and rh4-positive R7 cells12 (Figure 3). Therefore, proper coordination between R7 and R8 cells occurs through a directional R7-to-R8 signal: once an R7 cell makes a decision to express rh3 (e.g. to become a pR7 cell and to repress the default state of expressing rh4), it instructs an otherwise yR8 cell to ‘acquire’ a different cell fate and turn on rh5. While the nature of such a signal(s) is not known, this switch in fate likely involves the activation of rh5 as well as the repression of the default state, rh6, in order to avoid co-expression.

Exciting new data from Ng et al.37 has proposed just this type of on/off model for creating different cone cell populations in the mouse. Previous studies had demonstrated that treatment of retinal progenitor cells with thyroid hormone (T3) leads to a pronounced increase in cone cell differentiation.38 Furthermore, expression studies demonstrated that the Trβ2 isoform of the thyroid hormone receptor is highly enriched in the photoreceptor layer of the retina, suggesting a role in photoreceptor function and/or development.39 Indeed, the knockout of Trβ2 leads to a loss of M cone cells.37 However, these cells still develop into cone cells, but instead acquire an S cone cell fate. Thus, these data suggest that S cells represent a default differentiation pathway for cone cells and that M cells develop as a result of Trβ2-mediated activation of M cone opsin and/or repression of S cone opsin. Interestingly, several
studies focused on the timing of opsin expression have revealed a temporally-regulated replacement of S-to-M opsins, including humans (Reference 40 and references therein). These results suggest that S cones may represent a 'default' state in a wide variety of species, further supporting the idea of an S cell default state in these animals.

It is interesting to compare the default developmental pathways between vertebrates and flies. For instance, the default state in both systems represents the most abundant cell type and may employ very similar mechanisms. In mice, the ‘acquired state’, M cones, arise from the transformation of the S ‘default’ cones during development. In Drosophila, the choice between the ‘default’ and ‘acquired’ state is first made in R7 cells. As in vertebrates, it is likely that the ‘acquired’ pR7 cell arises from a ‘default’ yR7 cell. Unlike vertebrates, however, this acquisition needs not only to be maintained within the R7 cell, but must also be relayed to its underlying R8 cell in order to establish the coordinated expression of the p rhodopsins. Another distinction between vertebrates and Drosophila is the distribution of the default versus acquired state. In flies, the choice is stochastic, with no specific pattern for y or p ommatidium, whereas mammals appear to use spatial clues to regulate the same process. For instance, in humans, all types of cones are enriched in the fovea, with the S cells most often found on the outer perimeter of the fovea, while in mice, a dorso-ventral gradient of S cones is observed.\(^\text{1,8}\) Together, these results suggest that a diffusible signal controls the choice of cone cell fate. The new findings that Trβ2 participates in this process, together with the demonstration that T3 can induce cone cell differentiation in vitro, make it likely that hormones play an important role in cone subtype specification. The players involved in Drosophila subtype specification, however, remain to be identified.

**Role of opsins in morphogenesis**

Opsin expression is one of the latest events in photoreceptor differentiation, and the presence of a specific opsin is the primary molecular marker to distinguish among otherwise very similar cell populations. However, at least two other features exist to distinguish among different subpopulations of photoreceptors: their morphology and their connection to the neural network. For instance, in flies, the rhabdomeres of outer photoreceptors span the entire thickness of the retina, and their axons project to the proximal part of the optic lobe, the lamina. In contrast, the inner photoreceptor rhabdomeres lie one on top of the other, with the R7 occupying the apical portion of the retinal layer, and R8 located below R7 in the basal one-third of the retina. These cells have smaller rhabdomeres and their axons do not stop in the lamina, but instead project into the medullar region of the optic lobe.\(^\text{11}\)

Vertebrates have similar distinctions, with the cones and rods being morphologically distinguishable and each subtype projecting to different subsets of either bipolar or retinal ganglion cells for proper processing.\(^\text{41}\)

Several lines of evidence now exist that indicate that proper opsin gene expression is important to achieve proper photoreceptor differentiation. Rhodopsin mutations, for instance, lead to rod (or in fly, outer photoreceptor) degeneration and are responsible for a majority of cases of the most common retinal degenerative disease in man, retinitis pigmentosa.\(^\text{42,43}\) Other inherited forms of retinopathies, including cone–rod dystrophy, blue cone monochromacy, and Leber congenital amaurosis (a severe cone and rod degeneration of childhood onset) as well as additional cases of retinitis pigmentosa result from alterations in opsin gene regulation involving factors such as Crx and NRL.\(^\text{30,42,44,45}\) Importantly, misexpression of rhodopsin in rh-negative photoreceptors leads to rescue of the degeneration phenotype in both flies and mice.\(^\text{46,47}\) Moreover, this does not seem to be dependent on a specific rhodopsin molecule, as similar rescue of an rh1 mutation in flies is observed using Rh1, Rh3, Rh4, Rh5, or Rh6.\(^\text{48,49}\) These results suggest that a common feature among all rhodopsins allow these molecules to ‘catalyze’ rhabdomere formation. Indeed, a recent study by Don Ready’s laboratory has demonstrated that rh1 mutants could be rescued by activation of the actin-associated small GTPase, Rac1,\(^\text{50}\) demonstrating a direct signaling role of opsin that is distinct from its role in light transduction.

Based on the importance of rhodopsin in photoreceptor morphogenesis, the question arises as to whether opsins are sufficient to induce specification of a particular subtype of photoreceptor. In a different sensory system, the olfactory system, the expression of specific olfactory receptors is cell-type specific, mutually exclusive, and is linked to projections to defined glomeruli in the olfactory bulb. Interestingly, misexpression of one olfactory
receptor by the promoter of another leads to re-routing of projections. These studies implicate the olfactory receptor itself in instructing the cell to project to the correct position.

Although this question has not been addressed in vertebrates, it is unlikely that rhodopsins direct photoreceptor axon projections in flies. Several studies have demonstrated that very early in photoreceptor development, outer and inner photoreceptors project to their correct position in the optic lobe, indicating that a distinction between these two subtypes has been made long before the onset of rh expression (see References 11,52,53). However, a recent study by Mollereau et al.54 has revealed that further distinctions between inner and outer photoreceptors are controlled at much later developmental stages. In this study, the investigators demonstrated that mutation of the Spalt zinc finger transcription factor complex leads to late transformation of inner photoreceptors into outer photoreceptors. This transformation was observed by the loss of inner rh expression and a concomitant gain of rh1 expression together with the acquisition of outer photoreceptor rhabdomere shape and position within the ommatidia. However, these cells maintain their inner photoreceptor projections to the medulla, demonstrating that this change in fate occurs after axonal projection. Because Spalt expression is not observed in inner photoreceptors until approximately the same time as rh gene expression, it is possible that the mechanisms involved in Spalt-mediated processes may also participate in cell-specific decisions underlying p and y specification, including rh regulation. Thus, future work aimed at defining Spalt downstream effectors is likely to provide important insight into late developmental processes leading to subtype specification.

Summary

The past two decades have contributed a great deal to our understanding of photoreceptor differentiation. From these studies, it is clear that opsin expression is not only important for allowing photoreceptors to achieve their final role of absorbing light, but also may contribute to their final morphological features. Work on understanding the regulation of opsin gene expression indicates that regulation of these related genes occurs as a dual mechanism: photoreceptor specificity and subtype specificity. Subtype restriction occurs transcriptionally and generally involves a photoreceptor-specific element together with additional upstream sequences. In addition, humans have developed a further level of regulation through the use of an LCR. Interestingly, many of the factors identified thus far for regulating subtype specificity have a widespread distribution in photoreceptors, indicating that several layers of complexity are involved in controlling opsin gene expression. One mechanism appears to include a bifunctional role for some of these ubiquitous transcription factors. Otd, for instance, is expressed in all photoreceptors and can either activate or repress different subtypes of rhs in Drosophila and this activity is likely to involve interaction with cell-specific factors. Another mechanism that has emerged in recent years is communication between different photoreceptors. This was demonstrated by the findings that a developmental fate restriction achieved in R8 cells in response to the loss of R7 cells is as well as the recent demonstration that loss of Trβ2 leads to the conversion (or reversion) of cells to an S fate. Ongoing studies in this field of research are likely to provide us with information that is not only important for understanding how the visual system is built and maintained, but may also lend insight into the biological pathways devised in Nature to expand the repertoire of diverse photoreceptors.

Acknowledgements

We wish to thank Nadean Brown, Isabelle Brun, Brian Gebelein, Bertrand Mollereau, Maureen Neitz, and Franck Pichaud for their useful comments, suggestions, and discussion of this topic. We also thank Remi Sonneville for the image of the Rh5/Rh6 expression pattern shown in Figure 1. We apologize to those whose work has contributed to our understanding of photoreceptor subtype specification but was not included due to space limitations. TC is supported by an NIH NRSA fellowship. This work was supported by grants from NEI/NIH to CD.

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