Published in final edited form as:

Brain Res. 2008 February 4; 1192: 114–133.

Regulation of photoreceptor gene expression by Crx-associated transcription factor network

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Abstract

Rod and cone photoreceptors in the mammalian retina are special types of neurons that are responsible for phototransduction, the first step of vision. Development and maintenance of photoreceptors require precisely regulated gene expression. This regulation is mediated by a network of photoreceptor transcription factors centered on Crx, an Otx-like homeodomain transcription factor. The cell type (subtype) specificity of this network is governed by factors that are preferentially expressed by rods or cones or both, including the rod-determining factors neural retina leucine zipper protein (Nrl) and the orphan nuclear receptor Nr2e3; and cone-determining factors, mostly nuclear receptor family members. The best-documented of these include thyroid hormone receptor $\beta 2$ (Tr $\beta 2$), retinoid related orphan receptor Ror β , and retinoid X receptor Rxr γ . The appropriate function of this network also depends on general transcription factors and co-factors that are ubiquitously expressed, such as the Sp zinc finger transcription factors and STAGA coactivator complexes. These cell type-specific and general transcription regulators form complex interactomes; mutations that interfere with any of the interactions can cause photoreceptor development defects or degeneration. In this manuscript, we review recent progress on the roles of various photoreceptor transcription factors and interactions in photoreceptor subtype development. We also provide evidence of auto-, para-, and feedback regulation among these factors at the transcriptional level. These protein-protein and protein-promoter interactions provide precision and specificity in controlling photoreceptor subtype-specific gene expression, development and survival. Understanding these interactions may provide insights to more effective therapeutic interventions for photoreceptor diseases.

Keywords

Crx; retina development; cone and rod photoreceptors; transcription factor network; nuclear receptor; homeodomain

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FOOTNOTE REGARDING GENE NOMENCLATURE

Per guidelines of the Human Gene Nomenclature Committee, the names of the human genes and proteins are represented by capital letters, while only the first letter is capitalized for those from other species. Locus, gene and nucleotide sequence names are in italics and references to protein products are not italicized.

1. INTRODUCTION

Photoreceptors in the vertebrate retina carry out phototransduction, the conversion of light into a neuronal signal that initiates the visual process. In rodents, about 73% of retinal neurons are photoreceptor cells (Young, 1985). Rods and cones, the two types of photoreceptors in the retina, show a species-specific ratio and spatial distribution. Rods are responsible for vision in dim light, while cones are responsible for color vision in bright light. Both rods and cones have unique cellular structures called outer segments, containing highly compact membrane discs where the visual pigment opsins and other machinery involved in phototransduction are densely packed. At the molecular level, photoreceptor cells preferentially express a set of genes that are essential for their function, so called photoreceptor-specific genes. Mutations in many of the photoreceptor specific genes are known to cause retinal degeneration diseases in humans. [For reviews, see (Hartong et al., 2006); and Retnet: <http://www.sph.uth.tmc.edu/Retnet/>]. Furthermore, the expression levels of these photoreceptor genes need to be precisely regulated. Increased or decreased expression levels of a wild-type photoreceptor gene can also lead to photoreceptor degeneration (Olsson et al., 1992; Humphries et al., 1997).

Precisely regulated photoreceptor gene expression is also a driving force for photoreceptor development/differentiation. Lineage tracing and birth dating experiments demonstrated that all of the neuronal cell types in the retina are derived from a common multi-potent progenitor cell (Turner and Cepko, 1987; Wetts and Fraser, 1988). For photoreceptor cells, cones are usually born (exit from the mitotic cycle and commit to the photoreceptor lineage) earlier than rods. In rodents, cones are born on embryonic days E11.5-E18.5, while rods are born in a longer period from E12.5 to postnatal day 7 (P7) with a peak at P0 (Carter-Dawson and LaVail, 1979; Young, 1985). However, there is a significant delay, several days in rodents, for newly born photoreceptor precursors to begin expressing the specific type of *opsin* and other genes that confer the mature phenotype (Watanabe and Raff, 1990; Cepko, 1996). During this lag time, the photoreceptor precursors appear to be “plastic” and can be induced to differentiate into different photoreceptor subtypes, depending on intrinsic and extrinsic regulatory factors (Nishida et al., 2003; Cheng et al., 2006; MacLaren et al., 2006; Roberts et al., 2006). The intrinsic factors mainly consist of transcription factors of homeodomain, bZIP and nuclear receptor families. In this manuscript, we review the recent progress in understanding these photoreceptor transcription factors, provide some evidence for the presence of network interactions among the major players, and present a model of how these interactions determine photoreceptor gene expression and development.

Photoreceptor transcription factors are the transcription regulators preferentially expressed by post-mitotic photoreceptor precursors and/or mature photoreceptors. Table 1 lists the major factors that are known to be important for photoreceptor development and maintenance, mostly based on in vivo loss-of-function studies. It is well established that members of bHLH and homeodomain transcription factor families play important roles in specifying various neuronal cell types in the retina [for review, see (Hatakeyama and Kageyama, 2004; Yan et al., 2005)], including photoreceptor precursors. Recently, though, much progress has been made in elucidating the roles of members of the nuclear receptor, bZIP and homeodomain families of transcription factors in specifying rod and cone photoreceptor subtypes. Below we will focus on these new findings and discuss some key factors in detail.

1.1. Factors specifying the photoreceptor lineage - Otx2 and Crx

The role of Otx homeodomain transcription factors in eye development originally came from studies of *Drosophila* orthodenticle (Otd), a paired-type homeodomain protein that is required for the formation of anterior brain, eye and antenna in the fly (Finkelstein and Boncinelli, 1994). Subsequent studies showed that Otd plays an essential role in *Drosophila* photoreceptor development (Vandendries et al., 1996) by regulating the expression of *opsin* genes (Tahayato

et al., 2003). Mammals have three *Otd* orthologs, *Otx1*, *Otx2* and *Crx*, which is equivalent to *Otx5* in fish, amphibians and chick (Plouhinec et al., 2003). The function of these *Otd* orthologs has diverged over time with *Crx* dedicated specifically to the development and maintenance of retinal photoreceptors and pinealocytes in the pineal gland involved in circadian regulation (see below). In terms of the protein sequence, the three mammalian *Otd* homologs share 87–88% homology in the homeodomain near the N-terminus and high homology in several discrete regions in the C-terminal portion, including a glutamine-rich region and the Otx-tail [(Chen et al., 1997; Furukawa et al., 1997); Figure 1]. Their homeodomain belongs to the K50 (lysine at position 50) paired-like class, similar to that of *Drosophila* bicoid protein, which, based on structure and functional studies, prefers to bind to DNA motifs with TAATCC or TAAGCT sequences (Treisman et al., 1989; Furukawa et al., 1997; Baird-Titus et al., 2006). These sequence motifs are widely present in the promoter or enhancer regions of many photoreceptor genes, including the *opsin* genes (Chen and Zack, 1996; Furukawa et al., 1997; Yu et al., 2006).

1.1.1. *Otx2* specifies photoreceptor lineage by regulating the expression of *Crx* and other photoreceptor genes—

Otx2 is expressed in the forebrain and midbrain neuroepithelium during development, including the eye domain. During development and in adults, *Otx2* is expressed in several eye tissues, including neural retina and retinal pigmented epithelium (RPE) (Bovolenta et al., 1997). *Otx2* is known to be required for the development and maintenance of the RPE by regulating the expression of RPE-specific transcription factors and genes (Martinez-Morales et al., 2001; Martinez-Morales et al., 2003). In the neural retina, *Otx2* expression is seen in post-mitotic neuroblast cells that have the potential to develop into various cell types, including ganglion cells, bipolar cells and photoreceptor cells (Bovolenta et al., 1997; Baas et al., 2000). Nishida et al. carried out a parallel in situ hybridization analysis of *Otx2* and *Crx* mRNA expression in developing mouse retina (Nishida et al., 2003) and showed that *Otx2* expression is initially seen at E11.5. Its expression increases at E12.5 together with an induction of *Crx* expression, coinciding with early cone development. At E17.5, *Otx2* expression is highly intensified in the outer part of the neuroblastic layer, where a *Crx* expression zone is established. After birth, when *Crx* expression reaches a peak and rod maturation begins around P5–6, *Otx2* expression is down-regulated in the presumptive photoreceptor cell layer but up-regulated in the inner nuclear layer where bipolar and Muller glia cells are developing. The *Otx2* spatial and temporal expression patterns suggest that *Otx2* could play an essential role in photoreceptor development.

Human genetic studies: The human *OTX2* gene maps to 14q21-q22, in an interval associated with microphthalmia and pituitary insufficiency. Using a candidate gene approach, Ragge et al., (2005) identified eight heterozygous *OTX2* mutations from 333 patients with ocular malformations. The ocular phenotypes of these patients vary from severe bilateral anophthalmia to unilateral microphthalmia with Leber's congenital amaurosis (LCA). In vitro biochemical analysis (Ragge et al., 2005; Chatelain et al., 2006) suggests that these *OTX2* mutations are likely to cause disease by a loss-of-function (haplo-insufficiency) mechanism, as many of the mutations reduce the ability of *OTX2* to bind to DNA and/or activate the target gene promoter *RBP3* in transfected HeLa cells.

Animal studies: Homozygous *Otx2* knockout in the mouse is embryonic lethal due to defects in gastrulation and lack of rostral brain (Acampora et al., 1995; Matsuo et al., 1995; Ang et al., 1996). The heterozygous *Otx2* knockout mouse (*Otx2*^{+/-}) showed multiple ocular defects, including microphthalmia, hyperplastic retina and RPE, and lack of lens, cornea and iris (Matsuo et al., 1995). To understand the role of *Otx2* in photoreceptor development, Nishida et al (2003) generated a conditional *Otx2* knockout in developing photoreceptors using a *Cre* transgene under control of the mouse *Crx* promoter. This *Otx2* deficiency converts developing

photoreceptors into amacrine-like cells in the retina, and completely blocks the formation of pinealocytes in the pineal gland. Thus, *Otx2* is required for photoreceptor cell fate determination and pineal gland development. *Otx2*'s role in photoreceptor development is also demonstrated by *Otx2* over-expression studies (Nishida et al., 2003). Forced *Otx2* expression in P0 rat retina using a retroviral vector results in a significant increase in the number of rod photoreceptors at the expense of bipolar, amacrine and Muller glia cells, suggesting that *Otx2* promotes photoreceptor cell fate (Nishida et al., 2003). Given the observation that *Otx2* expression switches to bipolar cells after the peak of photoreceptor development, these results also suggest that *Otx2* may be involved in bipolar development as well. Forced *Otx2* expression in adult iris- and ciliary-derived “stem” cells of rat origin is sufficient to induce the differentiation of photoreceptor-like cells (Akagi et al., 2004), consistent with *Otx2* having a key role in specifying photoreceptor cell fate.

Mechanisms of action: *Otx2* target genes in the photoreceptors are being studied by microarray analysis in Dr. Furukawa's laboratory in Japan. Although the microarray results remain to be seen, two direct *Otx2* target genes are known. One is *Rbp3* (Bobola et al., 1999; Fong and Fong, 1999), a *Crx*-independent gene (Furukawa et al., 1999) expressed by both rods and cones, and the other is *Crx*. *Crx* expression is abolished in the *Otx2* conditional knockout mouse retina (Nishida et al., 2003). *Otx2* significantly enhances *Crx* promoter activity in transient cotransfection assays. Chromatin immunoprecipitation analysis showed that *Otx2* binds to the promoter region of *Crx* in vivo, further supporting the *Crx* gene as a direct target of *Otx2* (see Results, Figure 3). *Otx2* has also been reported to bind to and auto-activate its own promoter (Martinez-Morales et al., 2003). Furthermore, we have shown that *Otx2* also binds to the promoter/enhancer region of several other known *Crx* targets, including rod and cone *opsins*, in the presence or absence of *Crx*. In transiently transfected HEK293 cells, *Otx2* is also able to activate *rhodopsin* and *M-cone opsin* promoter activity, although less potently than *Crx* (Peng and Chen, 2005). These results suggest that *Otx2* acts by directly regulating the expression of the key transcription factor *Crx* and its target genes.

1.1.2. *Crx* directly regulates the expression of many photoreceptor genes—*Crx* was identified by three laboratories independently. Using RT-PCR with degenerate primers corresponding to the paired-like homeodomain, Furukawa et al. cloned a murine *Crx* gene, which shows a photoreceptor-specific expression pattern (Furukawa et al., 1997). Chen et al. reported cloning bovine *Crx* using a yeast one-hybrid assay with a *rhodopsin* promoter element, Ret4, as bait (Chen et al., 1997) and demonstrated that *Crx* can bind to three target sites in the *rhodopsin* promoter as well as targets in several other rod gene promoters. Furthermore, *Crx* acts as a transcription activator and synergizes with the bZIP transcription factor Nrl in activating *rhodopsin*-reporter gene expression, suggesting for the first time that a high level of *rhodopsin* expression requires the function of at least two photoreceptor transcription factors. Using in situ hybridization analysis, *Crx* expression was found in both rods and cones, and in their precursors, starting at embryonic day 12.5 in mouse, coinciding with cone cell birth. Expression peaks at P5, correlating with the onset of rod photoreceptor maturation when rod-specific gene expression is turned up. BrdU incorporation assays confirmed that *Crx* expressing cells are post-mitotic photoreceptor precursors derived from those cells that have just exited the cell cycle and express *Otx2* but not *Pax6* (Garelli et al., 2006). *Crx* is the earliest expressed photoreceptor marker in the retina. It is also expressed in pinealocytes in the pineal gland and regulates photoentrainment (Furukawa et al., 1999) and expression of genes involved in synthesizing the circadian hormone melatonin in mice (Li et al., 1998). Interestingly, using immunohistochemistry studies, we have also found that *Crx* is expressed in rod bipolar cells that co-stain with the bipolar cell marker PKC α in both mouse and human retinas [(Wang et al., 2002); and data not shown], suggesting a possible role of *Crx* in bipolar cell function.

Human genetic studies: The first piece of evidence for Crx's role in development and maintenance of photoreceptors came from genetic studies performed by Freund et al. (1997), who cloned the human *CRX* gene based on its homology in the homeodomain to another retinal homeodomain protein, Chx10. The human *CRX* gene maps to 19q13.3, within a cone-rod dystrophy (CORD2) locus. Subsequent genetic screens not only identified *CRX* mutations in autosomal dominant cone-rod dystrophy (Freund et al., 1997), but also in autosomal dominant retinitis pigmentosa (adRP) (Sohocki et al., 1998) and Leber's congenital amaurosis (LCA) (Freund et al., 1998; Rivolta et al., 2001b). Most *CRX* mutations are inherited in an autosomal dominant manner or occur de novo, particularly in LCA cases (Rivolta et al., 2001a). Many mutations are nucleotide insertions or deletions resulting in formation of a premature stop codon 3' of the mutated sites, which produce C-terminal truncated forms of CRX. Others are missense mutations, several of which are located in the homeodomain [(Rivolta et al., 2001a); see Figure 1]. In vitro functional analysis demonstrated that many of the disease-linked mutations altered the ability of CRX to bind to DNA (homeodomain mutations) and/or activate transcription of the *rhodopsin* gene (Chen et al., 2002). Thus, *CRX* mutations may cause disease by impairing CRX-mediated transcriptional regulation of photoreceptor genes. However, in vitro biochemical studies have not found a clear correlation between disease severity and the degree of biochemical abnormality, and it is not clear why *CRX* mutations cause dominant disease.

Animal studies: The second piece of evidence for Crx function came from knockout mouse studies. Homozygous *Crx* knockout mice (*Crx*^{-/-}) are blind at birth without detectable photoreceptor function, resembling the phenotype of LCA. Their photoreceptors never develop the outer segments critical for phototransduction, and subsequently degenerate (Furukawa et al., 1999). Serial analysis of gene expression (SAGE) performed on *Crx*^{-/-} retinæ before the onset of photoreceptor degeneration showed that 46% of photoreceptor-enriched genes are Crx-dependent (Blackshaw et al., 2001), particularly the *opsin* genes, providing convincing evidence that altered photoreceptor gene expression is a primary cause of the Crx deficient phenotype. Heterozygous *Crx* knockout (*Crx*^{+/-}) mice, on the other hand, have normal photoreceptor function at the ages of 3 months or older. However, a delay in development of photoreceptor function was detected by electroretinogram (ERG) measures, despite normal appearance of the retina at one month of age (Furukawa et al., 1999). No photoreceptor degeneration was observed in *Crx*^{+/-} mice, raising the possibility that human diseases associated with *CRX* mutations could involve a dominant-negative effect on the Crx regulatory pathway.

The third piece of evidence for Crx function came from ectopic expression studies. Forced expression of recombinant Crx in P0 rat retina using a retroviral vector (Furukawa et al., 1997) induces rod differentiation, although less potent than forced Otx2 expression. As observed with Otx2, forced Crx expression leads to a reduction in the number of amacrine and Muller glia cells. However, the number of bipolar cells is unchanged. These results suggest that, like Otx2, Crx is an important factor for photoreceptor cell fate determination. In stem cell studies, forced *Crx* expression in adult rat iris- and ciliary-derived cells is sufficient to induce the formation of rhodopsin-expressing cells as potently as Otx2 (Haruta et al., 2001; Akagi et al., 2004). Similar experiments with primate stem cells, however, require both *Crx* and *NeuroD1* to induce the photoreceptor phenotype (Akagi et al., 2005). These findings suggest that interaction with other photoreceptor transcription factors is important for Crx function.

Mechanisms of action: Crx is a trans-activator for many photoreceptor genes, based on gene expression profile studies. Using chromatin immunoprecipitation assays, we have shown that Crx activates transcription by directly binding to the promoter and/or enhancer regions of the target genes in photoreceptor cells (Peng and Chen, 2005). However, in transient cell

transfection assays with target promoter-luciferase reporters, Crx alone has only a moderate transactivating activity (2–5-fold enhancement), even with the *rhodopsin* promoter, a well-known Crx target (Chen et al., 1997). Thus, one mechanism for Crx to activate transcription is to interact with other transcription regulators. Functional interactions with numerous other proteins have been reported. These include photoreceptor-specific transcription factors [Nrl and Nr2e3, discussed below; Qrx (Wang et al., 2004)] and general transcription factors [Sp family members (Lerner et al., 2001); and nuclear receptor Ror isoforms (Srinivas et al., 2006)]. Crx also interacts with chromatin remodeling factors [ataxin-7 (La Spada et al., 2001), HMG I/Y (Chau et al., 2000), Baf (Wang et al., 2002)], the transcription coactivators Cbp and p300 (Yanagi et al., 2000), and the STAGA coactivator/chromatin remodeling complex (Palhan et al., 2005). STAGA is a highly conserved multi-protein complex present from yeast (SAGA) to man (Martinez et al., 2001). One key component of STAGA is the histone acetyl-transferase (HAT) Gcn5 that catalyzes acetylation of histones, a chromatin modification often associated with transcriptional activation (Martinez et al., 2001). Crx interacts with STAGA via ataxin-7, a 110-kD protein in the STAGA complex. Expansion of the poly-glutamine tract of ataxin-7 is associated with a dominant neurological disorder, spinocerebellar ataxia type 7 (SCA7), which features cone-rod dystrophy-like retinal degeneration similar to the pathology linked to *CRX* mutations. Animal model studies and in vitro functional analysis suggest that Crx is a STAGA-dependent transcription activator (La Spada et al., 2001; Chen et al., 2004; Palhan et al., 2005). A polyglutamine-expanded ataxin-7 disrupts Gcn5 HAT activity, resulting in hypoacetylation of histones on Crx target genes and transcription impairment in a SCA7 transgenic mouse model. Thus, one possible mechanism for Crx transcription activation is to promote chromatin remodeling by recruiting STAGA or other HAT-containing coactivators to its target genes. This possibility is further supported by the findings that Crx also interacts with two other coactivators with intrinsic HAT activity, Cbp and p300 [(Yanagi et al., 2000); and data not shown].

One question related to Crx function is why the Crx deficient retina develops photoreceptor cells in the first place if Crx is critical for the expression of many photoreceptor genes. A possible explanation is that another Otd/Otx family member plays a redundant role with Crx in specifying photoreceptor cell fate, therefore partially compensating for Crx function in *Crx*^{-/-} mice. Indeed, the closely-related family member Otx2 is expressed in developing photoreceptors in the retina and up-regulated in the *Crx*^{-/-} mouse retina [(Furukawa et al., 1999); Table 2]. Apparently, Otx2 and Crx have redundant but indispensable roles in photoreceptor development and maintenance. These roles might be contributed by protein-protein or protein-promoter interactions between the two factors.

1.2. Factors for rod development - Nrl and Nr2e3

1.2.1. Nrl specifies rod lineage in photoreceptor precursors—The neural retina leucine zipper protein (Nrl) is a basic-leucine zipper (bZIP) transcription factor belonging to the Maf subfamily (Swaroop et al., 1992). The *Nrl* cDNA was originally cloned from an adult human retina library by subtractive hybridization (Swaroop et al., 1992). The recombinant Nrl protein was subsequently found to bind to and regulate the *rhodopsin* promoter via NRE, an AP-1 like element located in the proximal *rhodopsin* promoter region (Kumar et al., 1996; Rehmtulla et al., 1996). RT-PCR and in situ hybridization analysis demonstrated that *Nrl* is highly expressed in the retina, although expression was also detected in the developing brain and lens (Liu et al., 1996). In the neural retina, native *Nrl* transcripts were seen at E14.5 (Liu et al., 1996) and stay on through the developmental period and into adulthood. Lineage tracing using a *GFP* transgene driven by the *Nrl* promoter and BrdU pulse-chase experiments in mouse retina showed that *Nrl* mRNA can be detected as early as E12.5 in those cells just completing terminal mitosis (Akimoto et al., 2006). These *Nrl*⁺ cells subsequently develop into rod photoreceptors. In addition, *Nrl* is also highly expressed in the pineal gland of the brain

(Akimoto et al., 2006), suggesting a role in pineal gland development. At the protein level, Nrl in the nuclear fraction of human and mouse retinal extracts consists of multiple differentially phosphorylated isoforms ranging from 29 to 35 kDa on SDS-PAGE/Western blots (Swain et al., 2001; Kanda et al., 2007). The function of the phosphorylated Nrl isoforms remains to be determined, but they are more prominent at the peak of rod differentiation and are altered by some human *NRL* mutations [(Kanda et al., 2007); see below]. Immunostaining of Nrl in the human and mouse retina showed that Nrl is localized in the nucleus of rods but not cones (Swain et al., 2001). These results suggest that Nrl plays a role in rod development and maintenance.

Human genetic studies: The role of Nrl in rod function was first demonstrated by human genetic studies. The human *NRL* gene maps to chromosome 14q11.2 (Farjo et al., 1997). Subsequent mutation analysis of a large pedigree with autosomal dominant retinitis pigmentosa identified a S50T missense mutation in *NRL* that cosegregates with the disease (Bessant et al., 1999). Although *NRL* mutations are rare, additional missense mutations linked to adRP have been identified, with hot spots at residues S50 and P51 (Martinez-Gimeno et al., 2001; DeAngelis et al., 2002; Nishiguchi et al., 2004). Some of these hot spot mutations result in mutant forms of *NRL* that demonstrate reduced phosphorylation but hyperactivity in activating the *rhodopsin* promoter with CRX in vitro (Bessant et al., 1999; Nishiguchi et al., 2004; Kanda et al., 2007). This suggests that these are gain-of-function mutations. It is notable that the function of both rods and cones are more severely affected in patients with heterozygous *NRL* mutations than in patients with the *RHODOPSIN* mutation P23H (DeAngelis et al., 2002), raising the possibility that *NRL* mutations could actively affect cone function in humans. Recessive *NRL* mutations were also found in patients suffering clumped pigmentary retinal degeneration (Nishiguchi et al., 2004) with loss of rod, but normal blue cone function. These mutations are likely to be loss-of-function mutations as shown by in vitro function studies (Nishiguchi et al., 2004).

Animal studies: The most direct evidence for Nrl function in rod fate determination comes from *Nrl* knockout mouse studies (Mears et al., 2001). Knockout of *Nrl* results in loss of rod photoreceptor function based on ERG measures. However, the function of cones, especially S-cones, is significantly enhanced compared to that in wild-type mice, resembling the phenotype of enhanced S-cone syndrome (ESCS) in humans and rd7 in mice, both associated with *Nr2e3* mutations (see below). Cone function in *Nrl* knockout mice is preserved as late as 31 weeks (Mears et al., 2001). Single-cell electrophysiology showed responses driven by both S- and M-opsin in all cells tested from *Nrl* knockout mice (Nikonov et al., 2005), as seen for the wild-type cones that co-express both opsins [(Applebury et al., 2000; Nikonov et al., 2006); see *cone subtypes and gradients* section below]. Although the dorsal/ventral M/S-cone gradient is preserved in *Nrl*^{-/-} mice, more S-opsin sensitivity is seen in *Nrl*^{-/-} cones than in wild-type cones from comparable dorsal/ventral levels of the retina (Nikonov et al., 2006). Consistent with the above electrophysiological measures, the ultrastructural analysis showed that the *Nrl*^{-/-} retina has much shorter cone-like outer segments with disrupted morphology and cone-like nuclei with decondensed chromatin (Mears et al., 2001; Daniele et al., 2005). Whorls and rosettes are seen at early ages and thinning of the outer nuclear layer occurs later on (Mears et al., 2001), similar to retinal histopathology in rd7 mice. The inner neurons of the rod pathway, specifically rod bipolar cells, horizontal cells and amacrine cells, make connections with these “trans-differentiated” cones (Strettoi et al., 2004). At the molecular level, the *Nrl*^{-/-} retina has no detectable expression of rod-specific genes, but a much higher level of *S-opsin* and moderately increased *M-opsin* expression [(Mears et al., 2001); see Results and Table 2]. Thus, knockout of *Nrl* essentially turns a rod dominant retina into an all-cone (or rodless) retina based on morphological, physiological and molecular criteria, suggesting that Nrl is required for rod cell fate determination.

To determine if Nrl is sufficient to induce the rod phenotype, Oh et al. (2007) studied transgenic mice that express Nrl in all photoreceptor precursors under the control of a *Crx* promoter. This ectopic *Nrl* expression converts the retina of either wild-type (WT) or *Nrl*^{-/-} mice to an all-rod retina. The inner neurons that ordinarily contact cones, such as ON cone bipolar and horizontal cells, make connections with rods in these mice. No cone-specific gene products were detected by RT-PCR or immunohistochemistry (Oh et al., 2007). Thus, Nrl is sufficient to guide photoreceptor precursors to a rod lineage. However, conditional expression of *Nrl* later during S-cone differentiation, using the *S-cone* promoter on the *Nrl*^{-/-} background, generated hybrid cells that co-expressed both *rhodopsin* and *S-opsin*. Although lineage-tracing experiments showed that some of the S-cones might have switched to a rod lineage, no rod function was detected by ERG in these mice (Oh et al., 2007), suggesting that differentiated photoreceptors may have limited plasticity, but require appropriate transcriptional regulation for maintenance. Another possible explanation, however, is that Nrl represses expression of S-cone genes, resulting in inactivation of cells already committed to the S-cone lineage.

Mechanisms of action: Gene expression profile studies demonstrated that Nrl is required for the expression of many rod genes, including the photoreceptor nuclear receptor *Nr2e3* (Mears et al., 2001) that is linked to enhanced S-cone syndrome (ESCS) (see below). Thus, the phenotype of *Nrl*^{-/-} mice is in part contributed by the loss of *Nr2e3* expression. This was further demonstrated by mouse studies showing that ectopic expression of *Nr2e3* in photoreceptor precursors of *Nrl*^{-/-} mice can transform developing cones to rod-like photoreceptors (Cheng et al., 2006). Microarray analyses of the developing and mature retina of wild-type and *Nrl*^{-/-} mice have identified clusters of Nrl target genes (Yoshida et al., 2004; Yu et al., 2004; Akimoto et al., 2006). These not only include genes coding for phototransduction and structural or membrane associated proteins as expected, but also transcriptional regulators, intracellular transport proteins and components of known signaling pathways. The Bmp/Smad pathway is repressed in the *Nrl*^{-/-} retina, as *Bmp* (*Bmp2*, 4 and *Bmpr1a*) and *Smad* (*Smad 1*, 5, and 4) genes are down regulated (Yu et al., 2004). Chromatin immunoprecipitation assays demonstrated that Nrl binds to the promoter of *Bmp2* and *Bmp4*, suggesting they are direct targets of Nrl. In contrast, many components of the Wnt/Ca²⁺ signaling pathway showed altered (either up- or down-regulated) expression in *Nrl*^{-/-} retina. These results suggest that Nrl is also important for maintaining rod function and homeostasis by integrating various signaling pathways.

In the *Nrl*^{-/-} mouse, the expression of all rod-specific genes is lost but cone genes are upregulated, suggesting that Nrl represses cone genes either directly or indirectly. Chromatin immunoprecipitation assays showed that Nrl can directly bind to cone gene promoters, including *S-opsin*, *M-opsin*, cone arrestin (*Arr3*), and the cone transcription factor *Trβ2* [(Peng and Chen, 2005; Oh et al., 2007) and Figure 3], suggesting that Nrl may directly regulate cone gene expression in rods. On the other hand, transient co-transfection assays with luciferase reporter constructs driven by either *M-cone* or *S-cone opsin* promoters showed that Nrl actually activates cone *opsin* promoters in vitro (Peng et al., 2005). It is known that the retinoid X receptor gamma (Rxy) that represses S-cone expression (see below) is upregulated in the *Nrl*^{-/-} mouse retina. Furthermore, the expression of cone genes is upregulated in rd7 mouse retina where *Nr2e3* protein is missing, but Nrl is normally expressed (Peng et al., 2005). These findings suggest that Nrl itself may not act as a transcription repressor but rather indirectly repress cone genes via the function of Rxy, *Nr2e3*, or other transcription repressors (see below).

Nrl is known to interact with several transcriptional regulators, in addition to the possibility of forming heterodimers with other bZIP family members expressed in the retina, such as Jun/ Fos family members (He et al., 1998). Nrl was the first protein identified that acts synergistically with *Crx* to activate the *rhodopsin* promoter (Chen et al., 1997; Mitton et al.,

2000). This synergy, however, is not observed for cone *opsin* promoters (Peng and Chen, 2005). Nrl and Crx interact through the leucine zipper and homeodomain, respectively (Mitton et al., 2000). Two mutations in the CRX homeodomain identified in human patients (R41W and R90W) decreased this interaction (Mitton et al., 2000). Nrl also interacts with the TATA-binding protein, Tbp, through a different domain near the N-terminal that is conserved among Maf family members (Friedman et al., 2004). Thus, Nrl may function by recruiting or stabilizing Tbp, which then recruits the general transcription complex (Friedman et al., 2004). Nrl was also reported to interact with Fiz1, a zinc finger protein that complexes with Flt3 receptor tyrosine kinase (Mitton et al., 2003). Fiz1 potentiates Nrl or Crx/Nrl activity on *rhodopsin* and *PDE6B* promoters (Mali et al., 2007), further implicating Nrl's involvement in coordinating the intrinsic photoreceptor developmental program with extracellular signaling pathways.

1.2.2. Nr2e3 is a dual transcription regulator required for terminal differentiation of rods—The nuclear orphan receptor Nr2e3 (photoreceptor-specific nuclear receptor, PNR) was originally identified by its homology to the orphan nuclear receptor tailless (Tlx, Nr2e1) initially found in *Drosophila*, and by its specific expression in retinal photoreceptor cells (Kobayashi et al., 1999). Similar to other members of the Tlx nuclear receptor family, Nr2e3 has a zinc-finger DNA-binding domain near the N-terminus and a ligand-binding domain in the C-terminus for a ligand yet to be identified [(Kobayashi et al., 1999); Figure 1]. Nr2e3 is expressed in retinal photoreceptor cells beginning around E18 in the mouse, peaking during rod differentiation and persisting into adulthood at a decreased level (Kobayashi et al., 1999; Takezawa et al., 2007). Expression appears primarily localized to the outer nuclear layer (Kobayashi et al., 1999; Haider et al., 2000; Haider et al., 2001). This persistent expression, mainly in rods in mammals (Bumsted O'Brien et al., 2004; Chen et al., 2005; Peng et al., 2005), suggests that Nr2e3 plays a major role in rod differentiation and maintenance. On the other hand, some early cone precursors appear to transiently express Nr2e3, at least in lower vertebrates (Chen et al., 2005) and Nr2e3 expression has been reported in other retinal cell types (Chen et al., 1999) including mouse cones [(Haider et al., 2006); see below], so it may also be involved in other developmental processes.

Human genetic studies: Mutations in human *NR2E3* cause enhanced S-cone syndrome (ESCS), an autosomal recessive disease featuring hyperfunction of blue cones, defective function of rods, and blindness in the late stages (Haider et al., 2000; Jacobson et al., 2004; Wright et al., 2004). Histopathological studies showed excess S-cones in the retina, some of which express both *S-* and *M-cone opsins* (Milam et al., 2002), which is unusual in humans (Lukats et al., 2005; Peichl, 2005).

Animal studies: The rd7 mouse, a naturally occurring mutant strain, resembles the phenotype of human ESCS. Genetic analysis revealed that these mice carry a homozygous 380-bp deletion in the coding region of the *Nr2e3* cDNA (Akhmedov et al., 2000; Haider et al., 2001), due to mRNA splicing defects resulting from an L1-retrotransposon insertion (Chen et al., 2006). This leads to a frame-shift with a premature termination. Subsequent studies demonstrated that the rd7 mouse does not produce Nr2e3 protein, and therefore represents a bona fide null mutant of *Nr2e3* (*Nr2e3*^{-/-}) (Peng et al., 2005; Haider et al., 2006). The rd7 mouse retina contains whorls and rosettes in the photoreceptor layer at early ages, followed by slow photoreceptor degeneration. Similar to ESCS in humans, the rd7 retina has excess cones that express mostly S-cone opsin (Haider et al., 2001). Rod and cone function measured by electroretinography (ERG) is near normal in young adults but significantly declines in older mice as a result of degeneration of both rods and cones (Akhmedov et al., 2000; Ueno et al., 2005; Haider et al., 2006). These phenotypes of human ESCS and rd7 mice support a role for Nr2e3 in rod/cone development by demonstrating how its mutations lead to disease.

Mechanisms of action: The phenotype of *Nr2e3* mutants suggests two possible hypotheses for *Nr2e3* function in vivo: 1) *Nr2e3* mutations cause defects in cell-fate determination resulting in transdifferentiation of developing rods into cones, or 2) *Nr2e3* mutations result in abnormal cone proliferation leading to an increase in the absolute number of photoreceptors as well as disrupting the rod/cone ratio. Several pieces of evidence strongly support the first hypothesis: a) As a downstream target of the rod specific transcription factor Nrl, *Nr2e3* is predominantly expressed in post-mitotic developing and mature rods. In *Nrl*^{-/-} mice, enhanced S-cones arise from postmitotic rod precursors and *Nr2e3* expression is completely abolished (Mears et al., 2001). b) Morphological and microarray analysis of the rd7 retina indicate that the majority of photoreceptors exhibit a hybrid rod-cone phenotype, i.e. expressing both rod and cone genes (Chen et al., 2005; Corbo and Cepko, 2005). c) Direct target gene studies suggest that *Nr2e3* is a dual transcription regulator for both rod and cone genes. In vitro protein-DNA binding assays initially showed that *Nr2e3* recognizes a consensus DNA sequence with a direct repeat and binds as a homodimer (Kobayashi et al., 1999; Chen et al., 2005). This binding appears to mediate transcriptional repression (Chen et al., 2005) rather than activation as previously observed for the *rhodopsin* promoter (Cheng et al., 2004). The consensus *Nr2e3* DNA recognition sequence has actually not been found in native *opsin* promoters or other known target genes, although the half site is present (Peng et al., 2005). Chromatin immunoprecipitation assays subsequently demonstrated that the *Nr2e3* protein is indeed associated with both rod and cone *opsin* gene promoters in the retina of wild-type and “coneless” mice (Peng et al., 2005; Peng and Chen, 2005). However, this association depends on the Crx protein, as it does not occur in the *Crx*^{-/-} retina in spite of the presence of *Nr2e3* protein (Peng et al., 2005). Furthermore, in transient transfection assays, *Nr2e3* alone has limited regulated activity on target *opsin* gene promoters. In the presence of Crx and Nrl, however, *Nr2e3* potentiates Crx/Nrl activation of *rhodopsin* (Cheng et al., 2004; Peng et al., 2005), but represses their activity on the cone *opsin* promoters (Chen et al., 2005; Peng et al., 2005). In addition, four genetically identified *NR2E3* missense mutations demonstrated altered dual regulatory activity (Peng et al., 2005). Consistent with these in vitro results, quantitative RT-PCR analysis demonstrated that the rd7 retina exhibits down-regulated rod gene expression, but up-regulated cone gene expression during photoreceptor differentiation (Peng et al., 2005). These suggest that *Nr2e3* acts as a dual regulator to promote rod phenotype differentiation and suppress cone gene expression in developing rods by modulating Crx/Nrl activity on rod and cone promoters. d) Gain-of-function studies have shown that ectopic expression of *Nr2e3* using a *Crx* promoter in *Nrl*^{-/-} retina is sufficient for guiding photoreceptor precursors to develop into rod-like cells that express *rhodopsin* but not cone *opsins* (Cheng et al., 2006). Ectopic expression of *Nr2e3* driven by the *S-opsin* promoter is also sufficient to transform differentiating S-cones into rod-like cells. Altogether, these findings suggest that *Nr2e3* acts downstream of Nrl and Crx to reinforce the development of the rod phenotype by suppressing the cone pathway.

These findings, however, do not necessarily rule out the second possibility that *Nr2e3* has a function in limiting proliferation of early S-cone precursors (Haider et al., 2001; Yanagi et al., 2002). As seen in zebrafish retina (Chen et al., 2005), using more sensitive immunostaining assays with GFP-labeled cones, *Nr2e3* was recently found to be expressed in developing and mature M/S-cones (Haider et al., 2006). Some *Nr2e3* positive cells in E18 mouse retina also express Ki67, a mitotic marker, suggesting *Nr2e3* is expressed in mitotic cells of the developing retina. BrdU incorporation assays in rd7 retina at late developmental stages, including P14 and P30, demonstrated prolonged proliferation of ectopic retinal progenitors that subsequently develop into S-cones. No S-cones were observed to re-enter the cell cycle, however. Increased apoptosis is also seen in late developing stages of rd7 retina. These results support a role of *Nr2e3* in suppressing cone proliferation.

The molecular mechanisms by which Nr2e3 plays a dual regulatory role in rod vs. cone gene expression remain to be determined, but are expected to involve interactions with co-activators and co-repressors. Nr2e3 is known to interact with another nuclear receptor, Nr1d1 (Rev-erb- α) (Cheng et al., 2004), forming a complex with Crx/Nrl that potentiates *rhodopsin* promoter activity. Nr1d1 is a member of the circadian clock (Yin et al., 2006) involved in regulating diurnal variations in gene expression. It is also known that the expression of some photoreceptor genes such as *rhodopsin* is under circadian regulation (Bowes et al., 1988; von Schantz et al., 1999). Thus the Nr2e3/Nr1d1 interaction may play a role in regulating the diurnal expression pattern of these genes. Nr2e3 has also been reported to interact with Rar α and Rxr β (Chen et al., 1999). As for possible co-repressors, its closely-related family member Nr2e1 (Tlx), was reported to interact with the co-repressor atrophin1 (Atn1) in the retina (Zhang et al., 2006), raising the possibility that Nr2e3 might also use Atn1 as a co-repressor. Interestingly, Nr2e1 plays a role in modulating retinal progenitor cell proliferation and cell cycle re-entry by inhibiting the expression of *Pten*, a negative regulator of neural stem cell proliferation (Zhang et al., 2006). An *Nr2e1* null mutation also results in enhanced S-cone syndrome in mice (Zhang et al., 2006). Takezawa et al. (2007) identified a novel cell cycle-dependent Nr2e3 co-repressor named Ret-CoR, which is preferentially expressed in the developing retina and brain as well as Y79 retinoblastoma cells. Ret-CoR expression is down regulated in mature retina, but clearly present in the photoreceptor nuclear layer where Nr2e3 is expressed. As reported for the other nuclear co-repressors NCoR/SMRT, Ret-CoR forms a multiprotein complex containing histone deacetylases (HDAC 1/2 and 3), NCoR and Rb/p107 that are known to regulate cell cycle progression. Nr2e3 appears to recruit Ret-CoR to the promoter of *Cyclin D1*, which is required for proliferation of retinal progenitor cells, and repress its expression. This new finding supports a possible role of Nr2e3 in regulating cell proliferation as discussed above. Altogether, these findings suggest that Nr2e3 promotes rod differentiation by bi-directionally regulating rod vs. cone gene expression and possibly inhibiting the proliferation of developing cones.

1.3. Factors for cone development

1.3.1. Cone subtypes and gradients—Cones are less sensitive to light than rods, but they provide visual acuity, the ability to distinguish features of the visual environment. Consequently, their distribution across the retina is not random, but varies among species to reflect each species' visual needs [(Peichl, 2005); Figure 2]. Most vertebrates have at least two different cone subtypes, producing opsins with different spectral sensitivities. In addition, the cone outputs are subjected to more processing in the retina than rod signals in order to extract more visual information, so cones make synaptic connections with a larger number and greater variety of inner retina cells than rods. Conversely, in order to preserve spatial resolution, cone signals are not summed by converging on a small number of output cells the way rod signals are (Peichl, 2005). The result is that more inner retina and central visual pathway neurons are involved with processing cone signals than rod signals. This has implications for understanding retina development, and should also be taken into account in experiments in which photoreceptor fate is genetically manipulated.

Most studies aimed at understanding the genetic mechanisms underlying human cone development have been performed in mice, which have two subtypes of cones. Mice do not have a cone-rich area in their retina like primates do, but the two cone subtypes are distributed in inverse gradients across the retina (Figure 2A-B). The density of cones expressing short-wavelength sensitive S-opsin is highest in the ventral retina, and cones expressing longer wavelength sensitive M-opsin are most dense in the dorsal retina. In the region where these two gradients overlap, many cones express both photopigments (Rohlich et al., 1994; Applebury et al., 2000; Lukats et al., 2005). As in most vertebrates, there is a region of higher visual sensitivity along the equator of the retina that corresponds with a disproportionately increased area of representation in the visual cortex (Luo et al., 2006). In the mouse retina, S-

cones differentiate first, beginning shortly after birth, with most differentiating cells localized to the ventral retina. M-cones begin differentiating about a week later, mostly in the dorsal retina and coinciding with a decrease in the number of cells expressing S-opsin (Cepko, 1996; Roberts et al., 2006). Increasing evidence suggests that the cone gradients are generated during development by the action of diffusible growth factors or hormones (extrinsic signals) and their receptors, mainly nuclear receptor family transcription factors (intrinsic program). These receptors exist as families of related genes that have apparently diverged from a common ancestral precursor (Germain et al., 2006). In the following section, we will focus on recent progress on the role of nuclear receptors in cone development and discuss the possible action of their ligands.

1.3.2. Factors that influence S-cone differentiation

Extrinsic signal – to be determined: The regulatory factors that are responsible for triggering cone progenitors to begin differentiating are still not well understood. Retinoic acid or related compounds are tempting candidates, since they have been shown to induce expression of photoreceptor specific genes and to influence cell fate choices in vitro (Hyatt and Dowling, 1997). It is also known that during early eye development in rodents, the equator region of the retina expresses retinoic acid degrading enzymes, while the rest of the retina expresses enzymes that convert Vitamin A to retinoic acid, thus establishing a discontinuous gradient of retinoic acid signaling across the retina (Luo et al., 2006). However, there is currently no compelling evidence that retinoic acid or related compounds influence cone photoreceptor distribution in vivo, although these studies led to interesting findings regarding the receptors for these compounds.

Rxry and Trβ2 are negative regulators for S-cones: Retinoic acid and related compounds work by diffusing through cellular membranes and binding to nuclear receptors, which associate with specific regulatory elements in the DNA of gene promoter and enhancer regions. These receptors exist as families of related genes. The retinoic acid receptor (Rar) and retinoid-related receptor (Rxr) families both consist of three genes, *A*, *B*, and *C*, producing the α , β , and γ isoforms, respectively, of the receptors. Extensive knockout mouse studies have been performed to try to determine which isoforms are expressed in particular tissues and mediate signals for particular processes or sets of genes. Most isoforms are expressed in the retina (Janssen et al., 1999; Mori et al., 2001), but the Rxry receptor is of particular interest because it is localized to developing cone photoreceptors in a number of species. Knockout of Rxry in mice destroys the gradient of S-cone distribution and results in S-opsin expression in all cones in the retina (Roberts et al., 2005), indicating that its likely role in S-cones is inhibitory rather than inductive. Rxrs are unique among nuclear receptors because they heterodimerize with members of several other nuclear receptor families, including Nr2e3 (Chen et al., 1999) and thyroid hormone receptors (Szanto et al., 2004). Thyroid hormone receptor Trβ2 is likely to be the heterodimerization partner involved in suppressing S-cones (see below).

Rorβ2 is a positive regulator for S-cones: Retinoid-related orphan receptors (Ror) are another family of genetically-related receptors with homology to the receptors for retinoids, but whose actual ligands have not been identified. One isoform in particular, Rorβ2, is expressed in photoreceptors as well as other cells in regions of the brain involved in regulating circadian rhythm (Andre et al., 1998). This isoform is expressed early in retinal progenitor cells (beginning at E13.5 in rat) and appears to increase their proliferation (Chow et al., 1998). The other product of this gene, Rorβ1, is not produced in the retina (Azadi et al., 2002). As development proceeds, expression of Rorβ2 continues at a lower level in photoreceptor cells, as well as in amacrine and ganglion cells (Chow et al., 1998). Rorβ2 has also been shown to synergize with Crx in vitro to activate the *S-opsin* gene (Srinivas et al., 2006). Mice that are homozygous for knockout of *Rorβ* fail to express S-opsin at the appropriate developmental

time, although M-opsin expression is unaffected. However, in these mice the outer nuclear layer is disorganized and photoreceptor outer and inner segments are not produced, indicating that *Rorb2* may have several additional roles in differentiation of both rod and cone photoreceptors (Srinivas et al., 2006). Yanagi et al. (2002) suggest that the S-cone phenotype is a default state, to explain why disruption of either rod-inducing factors (*Nrl* or *Nr2e3*) or factors involved in cone differentiation (*Rxry* or *Trb2*) result in over-production of S-cones. The discovery of this positive regulator of *S-opsin*, however, suggests that the S-cone phenotype might be actively selected, arguing against the hypothesis of a default pathway.

1.3.3. Factors that influence M-cone differentiation

Extrinsic signal – thyroid hormone: M-cones develop later than S-cones in rodents, but the mechanisms involved are currently more completely understood. The M-cone gradient (Figure 2A-B) is most likely established by thyroid hormone (TH). This hormone is produced by the thyroid gland and distributed by blood circulation to the tissues, where it is partially deiodinated to the active form (3,5,3' tri-iodothyronine, or T3) (Forrest et al., 2002). Its involvement in mediating the visual changes that occur during amphibian metamorphosis have long been known (Hoskins, 1990), and it was also shown to influence chick and rat retinal progenitor development. Harpavat and Cepko reviewed the role of TH in retinal development in 2003 (Harpavat and Cepko, 2003). TH is distributed uniformly across the retina at birth, but during the period of M-cone differentiation between P4 and P10 forms a gradient with higher concentrations in the dorsal than ventral retina [(Roberts et al., 2006); Figure 2B]. This implicates TH as the extrinsic signal responsible for establishing the M-cone gradient.

Trb2 is a positive regulator for M-cones: Vertebrates have two genes for TH receptors, and each produces several protein isoforms [reviewed in (Forrest et al., 2002; Eckey et al., 2003)]. The thyroid hormone receptor *Trb2*, a splice variant of the thyroid hormone receptor B (*Thrb*) gene, is implicated in photoreceptor development in chick and mouse, based on its expression in retinal progenitor cells and developing photoreceptors. In mouse eyes, expression of *Trb2* begins about E16, peaks around E18 as cone photoreceptors begin differentiating, then decreases (Ng et al., 2001; Yanagi et al., 2002), but the expression is uniform across the retina (Ng et al., 2001; Roberts et al., 2005; Roberts et al., 2006). However, during the time M-cones are developing, its ligand TH becomes distributed in a gradient with higher concentrations in the dorsal than ventral retina [(Roberts et al., 2006); Figure 2B].

In vitro and animal studies: Transient transfection studies showed that *Trb2*, in the presence of TH, activated *M-opsin* transcription and inhibited Crx-mediated transcription of *S-opsin* (Yanagi et al., 2002). Addition of exogenous TH also promoted *M-opsin* expression and inhibited *S-opsin* in embryonic retina explant cultures from wild-type (WT) but not *Trb2* knockout mice (Roberts et al., 2006). Furthermore, daily injection of TH into WT mouse pups beginning on P0 drastically decreased the number of S-cones found in all parts of the retina three days later. No decrease in S-cone numbers was seen in *Trb2* knockout mice treated similarly (Roberts et al., 2006). These results showed that *Trb2* induced M-opsin expression and concurrently inhibited S-cone production in a ligand-dependent manner. Knockout of the photoreceptor-specific *Trb2* isoform of the *Thrb* gene converted all cones to the S-phenotype, resulting in loss of both M-opsin expression and the S-cone gradient in vivo (Ng et al., 2001). This phenotype is also reproduced in a mouse with a knockin mutation in the DNA binding domain of *Thrb* that abolishes specific DNA sequence binding without affecting ligand binding or cofactor interactions (Shibusawa et al., 2003). These findings indicate that both M-opsin induction and the establishment of the S-cone gradient depend on *Trb2* DNA binding. The role of *Trb2* thus appears to be to induce a subset of developing cones to further specialize as M-cones (Yanagi et al., 2002) by suppressing expression of *S-opsin* (and possibly other S-cone genes) but promoting expression of *M-opsin* (and possibly other M-cone genes).

Mechanisms of action: TH nuclear receptors are reported to exert their effects as heterodimers in combination with retinoid (usually Rxr) receptors (Mangelsdorf and Evans, 1995). The Rxry receptor mentioned previously is a likely candidate for the Tr β 2 dimerization partner in developing cones. Rxry itself is not involved in *M-opsin* expression, however, since the M-cone gradient forms normally in *Rxry* knockout mice (Roberts et al., 2005). Roberts et al. (Roberts et al., 2006) hypothesize that a Tr β 2:Rxry heterodimer is responsible for suppressing *S-opsin* expression, while binding of TH induces dissociation of Rxry and formation of Tr β 2 homodimers, which activate *M-opsin* expression. This would also explain the inhibition of *S-opsin* expression in the dorsal retina prior to P6. Exogenous TH could overcome this inhibition if it induced dissociation of receptor heterodimers prematurely, before the gradient of thyroid hormone becomes established. Thyroid hormone receptors mediate repression (usually in the absence of ligand) by interacting with nuclear co-repressors NCoR and/or SMRT (Eckey et al., 2003; Makowski et al., 2003; Havis et al., 2006). In fact, a growing body of evidence indicates that heterodimers of thyroid hormone and Rxr receptors adopt different configurations based on information provided by the DNA binding site with which they associate, that facilitate or block interactions with co-activators or co-repressors [(Harvey et al., 2007) and references cited therein; (Ghosh et al., 2002; Diallo et al., 2007)]. Thus, the action of a nuclear receptor heterodimer can be influenced by each target promoter sequence to fine-tune interactions with ligands and cofactors. Since S-cones develop several days before M-cones appear in mice but are less prevalent in the dorsal retina, a repressive mechanism must exist for suppressing S-cones (or at least expression of S-cone genes) in regions where M-cones will predominate. Increasing evidence therefore supports a dual role for Tr β 2, in conjunction with Rxry, in mediating this repression.

The actual role of Tr β 2 on *S-opsin* expression may be more complex than the simple inhibition implied above. Findings from a study designed to map expression quantitative trait loci (eQTL) in the rat showed that point mutations affecting a conserved serine and another residue in the N-terminal domain of Tr β 2 (Ser56Asn and His58Arg) correlated with decreases in *S-opsin* expression levels of as much as 30% in homozygotes (Scheetz et al., 2006). The affected residues fall within a poorly characterized ligand-independent transactivation domain that can interact with cofactors and is the target of post-translational modification in some nuclear receptors (Germain et al., 2006). Tr β 2 binds directly to the *S-opsin* promoter (Figure 3) and has been reported to interact with the basal transcription machinery as well as co-activators and co-repressors to exert complex regulatory effects on target genes (Eckey et al., 2003). The mutations identified by Scheetz et al. (2006) might therefore alter one or more of these interactions, making activation of the *S-opsin* gene less efficient in the presence of the mutated receptor.

2. RESULTS AND DISCUSSION

Photoreceptor transcription factors form a network to regulate the expression of themselves and each other

During the course of reviewing and studying the above rod and cone transcription factors, we have observed evidence of cross-talk and feedback regulation among these factors at the transcriptional level. We hypothesized that each of these factors regulates its own expression and that of the other factors by direct binding of the transcription factor protein to promoter elements in the DNA. Such interaction and feedback regulation could be important for regulating development and maintenance of each of the photoreceptor phenotypes. To test this hypothesis, we performed chromatin immunoprecipitation (ChIP) and quantitative RT-PCR analysis of five of these transcription factors: the photoreceptor lineage determinant Crx, rod-lineage determinants Nrl and Nr2e3, the cone determination factor Tr β 2, and the HLH factor NeuroD1 that has been shown to be important for photoreceptor survival (Morrow et al., 1999; Pennesi et al., 2003). Chromatin immunoprecipitation (ChIP) assays were performed on

the retinas of wild-type, *Crx*^{-/-}, *Nrl*^{-/-} and *Nr2e3*^{rd7/rd7} (labeled as “Nr2e3^{-/-}”) mice at the age of P14 when these factors are all expressed but before photoreceptor degeneration begins in the mutant mice. The ChIP results were analyzed using PCR with primers spanning the promoter region of each regulatory factor; their dependent target genes, rod and cone opsins; and their independent gene *Rbp3* (Figure 3). PCR assays with primers spanning 3' regions immediately downstream of each gene were also performed as controls for the transcription factors' binding specificity to the regulatory regions (data not shown). To correlate the ChIP results with transcriptional regulation, we also performed quantitative RT-PCR for each of the regulatory factors in the retinas of the four strains (Table 2). These results, combined with what we have learned from the literature, are discussed below.

2.1. Opposing regulation of subtype-specific genes—It is poorly understood how each photoreceptor subtype expresses the genes that determine its own identity but shuts off expression of genes specific to other subtypes. Figure 3 shows that in wild-type retinas (“WT” lanes) all five photoreceptor-specific transcription factors bind to both rod and cone target genes, regardless of subtype association. Each of the transcription factors assayed is found on the promoters of *rhodopsin* (*Rho*), cone *opsins* (*Sop* and *Mop*) and *Rbp3*, genes expressed in photoreceptors, but not the control gene *GluR6* that is not expressed in photoreceptors. This suggests that each subtype-specific factor could play opposing roles on the expression of its own subtype-specific genes vs. genes specific to other photoreceptor subtypes. The best-understood example of this is Nr2e3, which is known to activate *rhodopsin* but repress cone *opsin* genes (Cheng et al., 2004; Peng et al., 2005). This is reflected in Figure 3 in the “Nr2e3” panel by the presence of Nr2e3 on all three *opsin* promoters, and in Table 2 by a decrease in *Rhodopsin* expression (0.84 times WT) and increases in *S-* and *M-opsin* expression (1.23 and 1.19 times WT, respectively) in the *Nr2e3*^{-/-} mouse compared with WT. Similarly, *Nrl* also directly binds to both rod and cone gene promoters in rods (Figure 3, “Nrl” panel), although a conventional *Nrl* target binding site has not been reported in the cone *opsin* regulatory sequences. This binding is independent of *Crx*, since it still occurs in the *Crx*^{-/-} retina. Whether *Nrl* binds to cone promoters through interaction with other proteins or as a result of the presence of low affinity binding site(s) in the cone promoters remains to be determined. In any case, the presence of *Nrl* on these promoters suggests that *Nrl* is involved in regulating both rod and cone genes in the same cell (Peng and Chen, 2005). Consistent with this, in the *Nrl*^{-/-} retina *Rho* gene expression is decreased (0.07 times WT) while cone *opsin* gene expression is increased (2.14 and 1.17 times WT; Table 2). Thus, *Nrl* exerts opposing effects on rod and cone gene expression in vivo. However, its repressive effect on cone genes is likely mediated by indirect mechanisms, as *Nrl* (alone or in combination with *Crx*) does not repress *M-* or *S-opsin* promoter activation in transiently transfected HEK293 cells (Peng et al., 2005).

The cone transcription factor *Trβ2* binds to both *M-opsin* and *S-opsin* promoters in WT retinas (Figure 3 “Trβ2” panel) to activate *M-opsin* but repress *S-opsin* expression (Ng et al., 2001; Yanagi et al., 2002). *Trβ2* also binds to the *rhodopsin* promoter (Figure 3) and several other rod genes (data not shown) in a *Crx*-dependent manner, suggesting that it could also be involved in regulating (likely repressing) the expression of rod genes in cone cells. Although no rod abnormalities have been reported in several different genetically engineered mice with disruptions of *Thrb/Trβ2*, this would be an interesting hypothesis to test. The outcome is likely to depend on interactions with other nuclear receptors and the availability of ligands and cofactors.

2.2. Auto-, para- and feedback regulation—Figure 3 also shows that each transcription factor binds to its own promoter as well as those of the other regulators examined (“WT” lanes in each panel), suggesting that each factor regulates its own expression (auto-regulation), regulates the other factors acting in parallel or downstream (para-regulation), and feeds back regulatory information to the promoters of the upstream factors that induced it (feedback

regulation). The best example of this is *Crx*. First, the *Crx* protein directly binds to its own promoter [Figure 3 and (Furukawa et al., 2002)] and auto-activates its own expression. The strength of *Crx* activation depends on the amount of *Crx* present. In *Crx* knockout mice that have low (*Crx*^{+/-}) or no (*Crx*^{-/-}) *Crx* protein as a result of replacement of the *Crx* coding sequence of one or both alleles, respectively, transgenic *LacZ* reporter genes driven by *Crx* promoter sequences are only expressed at 57% or 31% of wild-type levels, respectively (Furukawa et al., 2002). Thus, auto-activation substantially increases expression levels. Second, *Crx* also binds to the promoter of other photoreceptor transcription factors *Nrl*, *Nr2e3*, *Trβ2*, *Rxry*, *Rorβ*, and *NeuroD1* (Figure 3 “*Crx*” panel) and regulates their expression in para-regulatory fashion [Table 2; (Furukawa, 1999; Blackshaw et al., 2001)]. In *Crx*^{-/-} mice, expression of the rod factors *Nrl* and *Nr2e3* is significantly reduced but not abolished (0.67 and 0.61 times WT; Table 2), consistent with the dramatic reduction in *rhodopsin* expression (0.12 times WT). *Crx* is also required for the expression of the M-cone factor *Trβ2* by binding to the promoter of the *Trβ2* gene (Figure 3). *Trβ2* transcription in the *Crx*^{-/-} retina is only half (Table 2) of the wild-type level, consistent with lack of *Trβ2* binding on target cone genes (Figure 3, “*Trβ2*” panel) and defective cone *opsin* transcription (Table 2) in the *Crx*^{-/-} retina. Likewise, *Crx* may activate the expression of *NeuroD1* by binding to its promoter (Figure 3), as *NeuroD1* expression is also decreased (0.75 of WT level) in *Crx*^{-/-} mice [Table 2 and (Blackshaw et al., 2001)]. *NeuroD1* has been implicated in the survival and maintenance of both rods and cones (Morrow et al., 1999; Pennesi et al., 2003), and it may play this role by regulating the expression of rod/cone genes and their transcription regulators as suggested by the results shown in Figure 3. Although the reduced (0.75 times WT; Table 2) levels of *NeuroD1* apparently do not dramatically affect its binding to target genes in the *Crx*^{-/-} mice (Figure 3, “*NeuroD1*” panel), our results suggest that insufficient *NeuroD1* might contribute to the photoreceptor degeneration in these mice. Interestingly, *Rxry* and *Rorβ2* mRNA levels are elevated in the *Crx*^{-/-} retina [Table 2 and (Blackshaw et al., 2001)]. This suggests that *Crx* plays a negative regulatory role on expression of these two factors, although the mechanism for this remains to be determined. Finally, *Otx2* has been shown to act upstream of *Crx* by binding to and activating the *Crx* promoter (Nishida et al., 2003; Nishida, 2005). *Crx* also binds to the *Otx2* promoter to repress rather than activate *Otx2* expression, since *Otx2* transcript levels are increased more than two fold in *Crx*^{-/-} mice (Table 2). This is consistent with the fact that *Otx2* expression is down-regulated in the photoreceptor cell layer when *Crx* expression reaches a high level during normal retinal development (Nishida et al., 2003). Thus, at the center of the photoreceptor transcription factor network, *Crx* regulates expression of itself (auto-regulation), other members acting downstream or in parallel (para-regulation) and its upstream inducer (feedback regulation).

Similar auto- and para-regulation may also apply to photoreceptor subtype-specific regulatory factors. For example, the rod factor *Nrl* binds to its own promoter and auto-activates it, as only 40% of WT reporter transcript levels are seen in *Nrl*^{-/-} mice carrying a transgene under the control of the *Nrl* promoter (Yoshida et al., 2004). Besides regulating the expression of *Nr2e3* in the rod pathway, *Nrl* also binds to the *Trβ2*, *Rxry*, and *Rorβ* promoters in the cone pathway (Figure 3). This likely results in repression of *Rxry*, and *Rorβ*, as they are increased in *Nrl*^{-/-} retinas [Table 2 and (Yoshida et al., 2004)]. Although we did not detect significant changes in *Trβ2* expression levels or in *Trβ2* protein binding to target promoters in *Nrl*^{-/-} or *Nr2e3*^{-/-} mice, under physiological conditions in the wild-type background *Nrl* could play a role in repressing *Trβ2* expression either directly or indirectly. For the cone pathway factors, *Trβ2* binds to its own and other cone and rod regulator genes, raising the possibility that it could also mediate repression of these regulators to reinforce the M-cone pathway. *Trβ2* also binds to the promoters of upstream regulators *Crx* and *Otx2*, which could mediate feedback regulation of these factors. It would be interesting to evaluate this hypothesis by examining expression of these upstream regulators in *Trβ2* knockout mice.

2.3. Protein-promoter interactions can be affected by protein-protein interactions and accessibility of individual promoters—

The ChIP results presented in Figure 3 and expression level data in Table 2 also suggest crosstalk between protein-promoter interactions and protein-protein interactions for the photoreceptor transcription factors. As an example, Nr2e3 binding to its target genes, including both *opsins* and transcription regulators, appears to depend on its interacting partner Crx, as this binding does not occur in *Crx*^{−/−} mice. This lack of Nr2e3 target binding cannot be fully explained by the moderate reduction in Nr2e3 expression (60% of wild-type level) in *Crx*^{−/−} retina, as rd7 heterozygous mice that produce half the normal amount of Nr2e3 protein have normal Nr2e3 function. Furthermore, in homozygous *Crx*^{−/−} mice, Nr2e3 still binds to the Crx-independent gene *Rbp3*. Thus, Crx-dependent genes require either a high dose of Nr2e3 to bind to their promoters or else the presence of Crx to recruit Nr2e3 binding and regulation. Similarly, Trβ2 also appears to show such Crx-dependency in binding and regulating target genes (Figure 3, “Trβ2” panel), although no direct Trβ2/Crx interaction has been reported. In *Crx*^{−/−} mice, consistent with the reduction of Trβ2 mRNA shown in Table 2, the Trβ2 protein level is also moderately reduced on Western blots (data not shown). This reduction cannot fully account for the lack of Trβ2 binding to rod or cone target genes in *Crx*^{−/−} mice, suggesting that Trβ2 binding to target genes depends on Crx or other Crx-regulated factor(s). Since many nuclear receptor consensus binding sites in photoreceptor gene promoters appear not to favor binding of nuclear receptor homodimers, these results raised the possibility that other nuclear receptors known to regulate photoreceptor genes might also function in a similar way, i.e. interacting with Crx/Otx2 to bind and regulate photoreceptor genes. In contrast, Nrl does not appear to be required for binding of the nuclear receptors to photoreceptor genes, as Trβ2 binds to its targets well in *Nrl*^{−/−} mice (Figure 3). No Nr2e3 target binding is detected in *Nrl*^{−/−} mice because Nr2e3 is not made in this genetic background [(Mears et al., 2001); and Table 2]. We have not yet observed dramatic changes in target binding for Nrl and Crx in any of the mutant strains tested, suggesting their binding is independent of the other factors examined.

One exception for Crx-dependent binding of nuclear receptors to target genes is the *Rbp3* gene. *Rbp3* is known to be a target of Otx2 (Fong and Fong, 1999) and is expressed early during retina development, at the time *Crx* is turned on (Bibb et al., 2001). Thus, Otx2 might assist Nr2e3 and Trβ2 binding to the *Rbp3* promoter in *Crx*^{−/−} mice. Another possible explanation is that the *Rbp3* promoter is more accessible to regulatory factors so that a low concentration of a nuclear receptor in the presence of Otx2 is sufficient for *Rbp3* promoter binding. It would be interesting to compare histone modification and other chromatin configuration markers between the *Rbp3* promoter and the other promoters, to determine what makes *Rbp3* more accessible to regulatory factors.

3. CONCLUSION

Taken together, the recent progress in understanding the molecular mechanisms controlling photoreceptor subtype development and the results of the combined ChIP-expression analysis presented above suggest that the photoreceptor transcription factors form two types of network: protein-protein interaction and protein-promoter interaction. The information for these two networks is just beginning to emerge. Figure 4 shows our current model for the protein-promoter interaction network. Although this model is still missing components and connections, it does offer an overview of how this protein-promoter interaction network coordinates the auto-, para- and feedback regulation among photoreceptor transcription factors that determine general or subtype-specific photoreceptor lineages. This regulatory network is essential for precisely controlling spatial and temporal photoreceptor gene expression, development and maintenance. Therefore, perturbing any of the components, either by mutations or changes in expression levels of factors, could potentially disturb the balance of the network and result in developmental defects or degeneration of particular photoreceptor

subtypes. Understanding this network is important for future therapeutic interventions to treat those diseases associated with photoreceptor transcription factors.

4. EXPERIMENTAL PROCEDURES

Animals

All experimental procedures were pre-approved by the Institutional Animal Care and Use Committee of Washington University School of Medicine, and conformed to the guidelines of the Association for Research in Vision and Ophthalmology for the use of live animals in vision research. Mice were bred and maintained in barrier facilities at Washington University School of Medicine under a 12-hour light, 12-hour dark cycle. C57Bl/6J and rd7 mice were originally purchased from the Jackson Laboratory. *Nrl*^{-/-} mice were obtained from Anand Swaroop at the University of Michigan. *Crx*^{-/-} mice were kindly provided by Connie Cepko at Harvard Medical School.

Chromatin immunoprecipitation

The protocol used for chromatin immunoprecipitation has been published (Peng and Chen, 2005). Briefly, DNA and chromatin in pooled nuclear extracts from 6 retinæ were cross-linked with formaldehyde prior to immunoprecipitation with specific antibodies. Antibodies used in the work presented here that are not referenced in Peng and Chen (Peng and Chen, 2005) include: rabbit anti-Nrl [Chemicon; see (Swain et al., 2001) for specificity details], rabbit anti-Trβ2 [Upstate; see (Srinivas et al., 2006); and (Yen et al., 1992)] and goat anti-NeuroD1 [Santa Cruz sc-1084; see (Acharya et al., 1997); and (Cissell et al., 2003)]. Antibody specificity was confirmed by Western blotting and immunohistochemistry on retinal sections, comparing WT and the appropriate knockout mouse retinæ; or the use of antibodies from different sources, which gave identical results (data not shown). The results of ChIP assays were analyzed using candidate gene-based PCR with primers spanning the promoter region of each gene (listed in Peng et al., 2005 or shown in Table 3). PCR assays with primers spanning 3' regions immediately downstream of each gene were also performed as controls for factors' binding specifically in the regulatory regions (Peng and Chen, 2005). Results shown are representative of at least three separate experiments. Controls include the use of normal rabbit/goat IgG (Santa Cruz) in immunoprecipitation reactions (negative controls) and input (without ip) as positive controls in PCR reactions.

Quantitative real-time PCR

The protocol used for quantitative RT-PCR has been published (Peng and Chen, 2005). Sequences for additional primers used in this study are shown in Table 3. Briefly, cDNA reverse-transcribed from 1 µg total RNA was diluted 10-fold and quantified by real-time PCR analysis in triplicate on an iCycler PCR machine (Bio-Rad), using SYBR Green JumpStart ReadyMix (Sigma). *β-Actin* was used as a loading control. Relative expression levels were normalized to the *β-Actin* levels for each sample according to standard methodology (<http://www.openlink.org/dorak>), as follows: $\Delta CT = CT_{(test)} - CT_{(\beta-actin)}$ where CT, the threshold cycle, is the cycle number (in the exponential phase) at which enough amplified product has accumulated to yield a detectable fluorescent signal that is significantly above the baseline fluorescence level. Results are presented as the ratio of $\Delta CT_{knockout} / \Delta CT_{WT}$. Mean values and standard deviation (STDEV) were calculated for each experiment from three replicates, and statistical significance was determined using the paired student t-test.

ACKNOWLEDGEMENTS

The authors wish to thank Jianfeng Liu and Hui Wang for technical assistance. This work was supported by NIH grants EY12543 (to SC) and EY02687 (to Washington University Department of Ophthalmology and Visual Sciences Core) and an unrestricted grant from Research to Prevent Blindness, Inc.

LITERATURE REFERENCES

- Acampora D, Mazan S, Lallemand Y, Avantaggiato V, Maury M, Simeone A, Brulet P. Forebrain and midbrain regions are deleted in *Otx2*^{-/-} mutants due to a defective anterior neuroectoderm specification during gastrulation. *Development* 1995;121:3279–3290. [PubMed: 7588062]
- Acharya HR, Dooley CM, Thoreson WB, Ahmad I. cDNA cloning and expression analysis of *NeuroD* mRNA in human retina. *Biochem Biophys Res Commun* 1997;233:459–463. [PubMed: 9144558]
- Akagi T, Mandai M, Ooto S, Hirami Y, Osakada F, Kageyama R, Yoshimura N, Takahashi M. *Otx2* homeobox gene induces photoreceptor-specific phenotypes in cells derived from adult iris and ciliary tissue. *Invest Ophthalmol Vis Sci* 2004;45:4570–4575. [PubMed: 15557469]
- Akagi T, Akita J, Haruta M, Suzuki T, Honda Y, Inoue T, Yoshiura S, Kageyama R, Yatsu T, Yamada M, Takahashi M. Iris-derived cells from adult rodents and primates adopt photoreceptor-specific phenotypes. *Invest Ophthalmol Vis Sci* 2005;46:3411–3419. [PubMed: 16123446]
- Akhmedov NB, Piriev NI, Chang B, Rapoport AL, Hawes NL, Nishina PM, Nusinowitz S, Heckenlively JR, Roderick TH, Kozak CA, Danciger M, Davisson MT, Farber DB. A deletion in a photoreceptor-specific nuclear receptor mRNA causes retinal degeneration in the *rd7* mouse. *Proc Natl Acad Sci U S A* 2000;97:5551–5556. [PubMed: 10805811]
- Akimoto M, Cheng H, Zhu D, Brzezinski JA, Khanna R, Filippova E, Oh EC, Jing Y, Linares JL, Brooks M, Zarepari S, Mears AJ, Hero A, Glaser T, Swaroop A. Targeting of GFP to newborn rods by *Nrl* promoter and temporal expression profiling of flow-sorted photoreceptors. *Proc Natl Acad Sci U S A* 2006;103:3890–3895. [PubMed: 16505381]
- Andre E, Gawlas K, Becker-Andre M. A novel isoform of the orphan nuclear receptor ROR β is specifically expressed in pineal gland and retina. *Gene* 1998;216:277–283. [PubMed: 9729429]
- Ang SL, Jin O, Rhinn M, Daigle N, Stevenson L, Rossant J. A targeted mouse *Otx2* mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain. *Development* 1996;122:243–252. [PubMed: 8565836]
- Applebury ML, Antoch MP, Baxter LC, Chun LL, Falk JD, Farhangfar F, Kage K, Krzystolik MG, Lyass LA, Robbins JT. The murine cone photoreceptor: a single cone type expresses both S and M opsins with retinal spatial patterning. *Neuron* 2000;27:513–523. [PubMed: 11055434]
- Azadi S, Zhang Y, Caffé AR, Holmqvist B, van Veen T. Thyroid-beta2 and the retinoid RAR- α , RXR- γ and ROR- β 2 receptor mRNAs; expression profiles in mouse retina, retinal explants and neocortex. *Neuroreport* 2002;13:745–750. [PubMed: 11997680]
- Baas D, Bumsted KM, Martinez JA, Vaccarino FM, Wikler KC, Barnstable CJ. The subcellular localization of *Otx2* is cell-type specific and developmentally regulated in the mouse retina. *Brain Res Mol Brain Res* 2000;78:26–37. [PubMed: 10891582]
- Baird-Titus JM, Clark-Baldwin K, Dave V, Caperelli CA, Ma J, Rance M. The solution structure of the native K50 Bicoid homeodomain bound to the consensus TAATCC DNA-binding site. *J Mol Biol* 2006;356:1137–1151. [PubMed: 16406070]
- Bessant DA, Payne AM, Mitton KP, Wang QL, Swain PK, Plant C, Bird AC, Zack DJ, Swaroop A, Bhattacharya SS. A mutation in *NRL* is associated with autosomal dominant retinitis pigmentosa. *Nat Genet* 1999;21:355–356. [PubMed: 10192380]
- Bibb LC, Holt JK, Tarttlin EE, Hodges MD, Gregory-Evans K, Rutherford A, Lucas RJ, Sowden JC, Gregory-Evans CY. Temporal and spatial expression patterns of the CRX transcription factor and its downstream targets. Critical differences during human and mouse eye development. *Hum Mol Genet* 2001;10:1571–1579. [PubMed: 11468275]
- Blackshaw S, Fraioli RE, Furukawa T, Cepko CL. Comprehensive analysis of photoreceptor gene expression and the identification of candidate retinal disease genes. *Cell* 2001;107:579–589. [PubMed: 11733058]
- Bobola N, Briata P, Ilengo C, Rosatto N, Craft C, Corte G, Ravazzolo R. OTX2 homeodomain protein binds a DNA element necessary for interphotoreceptor retinoid binding protein gene expression. *Mech Dev* 1999;82:165–169. [PubMed: 10354480]
- Bovolenta P, Mallamaci A, Briata P, Corte G, Boncinelli E. Implication of OTX2 in pigment epithelium determination and neural retina differentiation. *J Neurosci* 1997;17:4243–4252. [PubMed: 9151741]

- Bowes C, van Veen T, Farber DB. Opsin, G-protein and 48-kDa protein in normal and rd mouse retinas: developmental expression of mRNAs and proteins and light/dark cycling of mRNAs. *Exp Eye Res* 1988;47:369–390. [PubMed: 2846333]
- Bumsted O'Brien KM, Cheng H, Jiang Y, Schulte D, Swaroop A, Hendrickson AE. Expression of photoreceptor-specific nuclear receptor NR2E3 in rod photoreceptors of fetal human retina. *Invest Ophthalmol Vis Sci* 2004;45:2807–2812. [PubMed: 15277507]
- Carter-Dawson LD, LaVail MM. Rods and cones in the mouse retina. II. Autoradiographic analysis of cell generation using tritiated thymidine. *J Comp Neurol* 1979;188:263–272. [PubMed: 500859]
- Cepko CL. The patterning and onset of opsin expression in vertebrate retinas. *Curr Opin Neurobiol* 1996;6:542–546. [PubMed: 8794108]
- Cepko C. Giving in to the blues. *Nat Genet* 2000;24:99–100. [PubMed: 10655045]
- Chatelain G, Fossat N, Brun G, Lamonerie T. Molecular dissection reveals decreased activity and not dominant negative effect in human OTX2 mutants. *J Mol Med* 2006;84:604–615. [PubMed: 16607563]
- Chau KY, Munshi N, Keane-Myers A, Cheung-Chau KW, Tai AK, Manfioletti G, Dorey CK, Thanos D, Zack DJ, Ono SJ. The architectural transcription factor high mobility group I(Y) participates in photoreceptor-specific gene expression. *J Neurosci* 2000;20:7317–7324. [PubMed: 11007889]
- Chen F, Figueroa DJ, Marmorstein AD, Zhang Q, Petrukhin K, Caskey CT, Austin CP. Retina-specific nuclear receptor: A potential regulator of cellular retinaldehyde-binding protein expressed in retinal pigment epithelium and Muller glial cells. *Proc Natl Acad Sci U S A* 1999;96:15149–15154. [PubMed: 10611353]
- Chen J, Rattner A, Nathans J. The rod photoreceptor-specific nuclear receptor Nr2e3 represses transcription of multiple cone-specific genes. *J Neurosci* 2005;25:118–129. [PubMed: 15634773]
- Chen J, Rattner A, Nathans J. Effects of L1 retrotransposon insertion on transcript processing, localization and accumulation: lessons from the retinal degeneration 7 mouse and implications for the genomic ecology of L1 elements. *Hum Mol Genet* 2006;15:2146–2156. [PubMed: 16723373]
- Chen S, Zack DJ. Ret 4, a positive acting rhodopsin regulatory element identified using a bovine retina in vitro transcription system. *J Biol Chem* 1996;271:28549–28557. [PubMed: 8910484]
- Chen S, Wang Q-L, Xu S, Liu Y, Lili YL, Wang Y, Zack DJ. Functional analysis of cone-rod homeobox (CRX) mutations associated with retinal dystrophy. *Hum Mol Genet* 2002;11:873–884. [PubMed: 11971869]
- Chen S, Peng GH, Wang X, Smith AC, Grote SK, Sopher BL, La Spada AR. Interference of Crx-dependent transcription by ataxin-7 involves interaction between the glutamine regions and requires the ataxin-7 carboxy-terminal region for nuclear localization. *Hum Mol Genet* 2004;13:53–67. [PubMed: 14613968]
- Chen S, Wang QL, Nie Z, Sun H, Lennon G, Copeland NG, Gilbert DJ, Jenkins NA, Zack DJ. Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. *Neuron* 1997;19:1017–1030. [PubMed: 9390516]
- Cheng H, Khanna H, Oh EC, Hicks D, Mitton KP, Swaroop A. Photoreceptor-specific nuclear receptor NR2E3 functions as a transcriptional activator in rod photoreceptors. *Hum Mol Genet* 2004;13:1563–1575. [PubMed: 15190009]
- Cheng H, Aleman TS, Cideciyan AV, Khanna R, Jacobson SG, Swaroop A. In vivo function of the orphan nuclear receptor NR2E3 in establishing photoreceptor identity during mammalian retinal development. *Hum Mol Genet* 2006;15:2588–2602. [PubMed: 16868010]
- Chow L, Levine EM, Reh TA. The nuclear receptor transcription factor, retinoid-related orphan receptor beta, regulates retinal progenitor proliferation. *Mech Dev* 1998;77:149–164. [PubMed: 9831642]
- Cissell MA, Zhao L, Sussel L, Henderson E, Stein R. Transcription factor occupancy of the insulin gene in vivo. Evidence for direct regulation by Nkx2.2. *J Biol Chem* 2003;278:751–756. [PubMed: 12426319]
- Corbo JC, Cepko CL. A hybrid photoreceptor expressing both rod and cone genes in a mouse model of enhanced S-cone syndrome. *PLoS Genet* 2005;1:e11. [PubMed: 16110338]
- Daniele LL, Lillo C, Lyubarsky AL, Nikonov SS, Philp N, Mears AJ, Swaroop A, Williams DS, Pugh EN Jr. Cone-like morphological, molecular, and electrophysiological features of the photoreceptors of the Nrl knockout mouse. *Invest Ophthalmol Vis Sci* 2005;46:2156–2167. [PubMed: 15914637]

- DeAngelis MM, Grimsby JL, Sandberg MA, Berson EL, Dryja TP. Novel Mutations in the NRL Gene and Associated Clinical Findings in Patients With Dominant Retinitis Pigmentosa. *Arch Ophthalmol* 2002;120:369–375. [PubMed: 11879142]
- Diallo EM, Wilhelm KG Jr, Thompson DL, Koenig RJ. Variable RXR requirements for thyroid hormone responsiveness of endogenous genes. *Mol Cell Endocrinol* 2007;264:149–156. [PubMed: 17161906]
- Eckey M, Moehren U, Baniahmad A. Gene silencing by the thyroid hormone receptor. *Mol Cell Endocrinol* 2003;213:13–22. [PubMed: 15062570]
- Farjo Q, Jackson A, Pieke-Dahl S, Scott K, Kimberling WJ, Sieving PA, Richards JE, Swaroop A. Human bZIP transcription factor gene NRL: structure, genomic sequence, and fine linkage mapping at 14q11.2 and negative mutation analysis in patients with retinal degeneration. *Genomics* 1997;45:395–401. [PubMed: 9344665]
- Finkelstein R, Boncinelli E. From fly head to mammalian forebrain: the story of otd and Otx. *Trends Genet* 1994;10:310–315. [PubMed: 7974744]
- Fong SL, Fong WB. Elements regulating the transcription of human interstitial retinoid-binding protein (IRBP) gene in cultured retinoblastoma cells. *Curr Eye Res* 1999;18:283–291. [PubMed: 10372988]
- Forrest D, Reh TA, Rusch A. Neurodevelopmental control by thyroid hormone receptors. *Curr Opin Neurobiol* 2002;12:49–56. [PubMed: 11861164]
- Freund CL, Wang QL, Chen S, Muskat BL, Wiles CD, Sheffield VC, Jacobson SG, McInnes RR, Zack DJ, Stone EM. De novo mutations in the CRX homeobox gene associated with Leber congenital amaurosis. *Nat Genet* 1998;18:311–312. [PubMed: 9537410]
- Freund CL, Gregory-Evans CY, Furukawa T, Papaioannou M, Looser J, Ploder L, Bellingham J, Ng D, Herbrick JA, Duncan A, Scherer SW, Tsui LC, Loutradis-Anagnostou A, Jacobson SG, Cepko CL, Bhattacharya SS, McInnes RR. Cone-rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (CRX) essential for maintenance of the photoreceptor. *Cell* 1997;91:543–553. [PubMed: 9390563]
- Friedman JS, Khanna H, Swain PK, Denicola R, Cheng H, Mitton KP, Weber CH, Hicks D, Swaroop A. The minimal transactivation domain of the basic motif-leucine zipper transcription factor NRL interacts with TATA-binding protein. *J Biol Chem* 2004;279:47233–47241. [PubMed: 15328344]
- Furukawa A, Koike C, Lippincott P, Cepko CL, Furukawa T. The mouse Crx 5'-upstream transgene sequence directs cell-specific and developmentally regulated expression in retinal photoreceptor cells. *J Neurosci* 2002;22:1640–1647. [PubMed: 11880494]
- Furukawa K. LAP2 binding protein 1 (LBP1/BAF) is a candidate mediator of LAP2-chromatin interaction. *J Cell Sci* 1999;112:2485–2492. [PubMed: 10393804]
- Furukawa T, Morrow EM, Cepko CL. Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* 1997;91:531–541. [PubMed: 9390562]
- Furukawa T, Morrow EM, Li T, Davis FC, Cepko CL. Retinopathy and attenuated circadian entrainment in Crx-deficient mice. *Nat Genet* 1999;23:466–470. [PubMed: 10581037]
- Garelli A, Rotstein NP, Politi LE. Docosahexaenoic acid promotes photoreceptor differentiation without altering Crx expression. *Invest Ophthalmol Vis Sci* 2006;47:3017–3027. [PubMed: 16799048]
- Germain P, Staels B, Dacquet C, Spedding M, Laudet V. Overview of nomenclature of nuclear receptors. *Pharmacol Rev* 2006;58:685–704. [PubMed: 17132848]
- Ghosh JC, Yang X, Zhang A, Lambert MH, Li H, Xu HE, Chen JD. Interactions that determine the assembly of a retinoid X receptor/corepressor complex. *Proc Natl Acad Sci U S A* 2002;99:5842–5847. [PubMed: 11972046]
- Haider NB, Naggert JK, Nishina PM. Excess cone cell proliferation due to lack of a functional NR2E3 causes retinal dysplasia and degeneration in rd7/rd7 mice. *Hum Mol Genet* 2001;10:1619–1626. [PubMed: 11487564]
- Haider NB, Demarco P, Nystuen AM, Huang X, Smith RS, McCall MA, Naggert JK, Nishina PM. The transcription factor Nr2e3 functions in retinal progenitors to suppress cone cell generation. *Vis Neurosci* 2006;23:917–929. [PubMed: 17266784]
- Haider NB, Jacobson SG, Cideciyan AV, Swiderski R, Streb LM, Searby C, Beck G, Hockey R, Hanna DB, Gorman S, Duhl D, Carmi R, Bennett J, Weleber RG, Fishman GA, Wright AF, Stone EM,

- Sheffield VC. Mutation of a nuclear receptor gene, NR2E3, causes enhanced S cone syndrome, a disorder of retinal cell fate. *Nat Genet* 2000;24:127–131. [PubMed: 10655056]
- Harpavat S, Cepko CL. Thyroid hormone and retinal development: an emerging field. *Thyroid* 2003;13:1013–1019. [PubMed: 14651785]
- Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet* 2006;368:1795–1809. [PubMed: 17113430]
- Haruta M, Kosaka M, Kanegae Y, Saito I, Inoue T, Kageyama R, Nishida A, Honda Y, Takahashi M. Induction of photoreceptor-specific phenotypes in adult mammalian iris tissue. *Nat Neurosci* 2001;12:12.
- Harvey CB, Bassett JH, Maruvada P, Yen PM, Williams GR. The rat thyroid hormone receptor (TR) {Delta}{beta}3 displays cell-, TR isoform- and thyroid hormone response element specific actions. *Endocrinology*. 2007
- Hatakeyama J, Kageyama R. Retinal cell fate determination and bHLH factors. *Semin Cell Dev Biol* 2004;15:83–89. [PubMed: 15036211]
- Havis E, Le Mevel S, Morvan Dubois G, Shi DL, Scanlan TS, Demeneix BA, Sachs LM. Unliganded thyroid hormone receptor is essential for *Xenopus laevis* eye development. *Embo J* 2006;25:4943–4951. [PubMed: 17006540]
- He L, Campbell ML, Srivastava D, Blocker YS, Harris JR, Swaroop A, Fox DA. Spatial and temporal expression of AP-1 responsive rod photoreceptor genes and bZIP transcription factors during development of the rat retina. *Mol Vis* 1998;4:32. [PubMed: 9873070]
- Hoskins SG. Metamorphosis of the amphibian eye. *J Neurobiol* 1990;21:970–989. [PubMed: 2175350]
- Humphries MM, Rancourt D, Farrar GJ, Kenna P, Hazel M, Bush RA, Sieving PA, Sheils DM, McNally N, Creighton P, Erven A, Boros A, Gulya K, Capocchi MR, Humphries P. Retinopathy induced in mice by targeted disruption of the rhodopsin gene [see comments]. *Nat Genet* 1997;15:216–219. [PubMed: 9020854]
- Hyatt GA, Dowling JE. Retinoic acid. A key molecule for eye and photoreceptor development. *Invest Ophthalmol Vis Sci* 1997;38:1471–1475. [PubMed: 9224274]
- Jacobson SG, Sumaroka A, Aleman TS, Cideciyan AV, Schwartz SB, Roman AJ, McInnes RR, Sheffield VC, Stone EM, Swaroop A, Wright AF. Nuclear receptor NR2E3 gene mutations distort human retinal laminar architecture and cause an unusual degeneration. *Hum Mol Genet* 2004;13:1893–1902. [PubMed: 15229190]
- Janssen JJ, Kuhlmann ED, van Vugt AH, Winkens HJ, Janssen BP, Deutman AF, Driessen CA. Retinoic acid receptors and retinoid X receptors in the mature retina: subtype determination and cellular distribution. *Curr Eye Res* 1999;19:338–347. [PubMed: 10520230]
- Kanda A, Friedman JS, Nishiguchi KM, Swaroop A. Retinopathy mutations in the bZIP protein NRL alter phosphorylation and transcriptional activity. *Hum Mutat* 2007;28:589–598. [PubMed: 17335001]
- Kobayashi M, Takezawa S, Hara K, Yu RT, Umesono Y, Agata K, Taniwaki M, Yasuda K, Umesono K. Identification of a photoreceptor cell-specific nuclear receptor. *Proc Natl Acad Sci U S A* 1999;96:4814–4819. [PubMed: 10220376]
- Kumar R, Chen S, Scheurer D, Wang QL, Duh E, Sung CH, Rehemtulla A, Swaroop A, Adler R, Zack DJ. The bZIP transcription factor Nrl stimulates rhodopsin promoter activity in primary retinal cell cultures. *J Biol Chem* 1996;271:29612–29618. [PubMed: 8939891]
- La Spada AR, Fu YH, Sopher BL, Libby RT, Wang X, Li LY, Einum DD, Huang J, Possin DE, Smith AC, Martinez RA, Koszdin KL, Treuting PM, Ware CB, Hurley JB, Ptacek LJ, Chen S. Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. *Neuron* 2001;31:913–927. [PubMed: 11580893]
- Lerner LE, Gribanova YE, Ji M, Knox BE, Farber DB. Nrl and Sp nuclear proteins mediate transcription of rod-specific cGMP-phosphodiesterase beta-subunit gene: involvement of multiple response elements. *J Biol Chem* 2001;276:34999–35007. [PubMed: 11438531]
- Li X, Chen S, Wang Q, Zack DJ, Snyder SH, Borjigin J. A pineal regulatory element (PIRE) mediates transactivation by the pineal/retina-specific transcription factor CRX. *Proc Natl Acad Sci U S A* 1998;95:1876–1881. [PubMed: 9465110]

- Liu Q, Ji X, Breitman ML, Hitchcock PF, Swaroop A. Expression of the bZIP transcription factor gene *Nrl* in the developing nervous system. *Oncogene* 1996;12:207–211. [PubMed: 8552394]
- Lukats A, Szabo A, Rohlich P, Vigh B, Szel A. Photopigment coexpression in mammals: comparative and developmental aspects. *Histol Histopathol* 2005;20:551–574. [PubMed: 15736061]
- Luo T, Sakai Y, Wagner E, Drager UC. Retinoids, eye development, and maturation of visual function. *J Neurobiol* 2006;66:677–686. [PubMed: 16688765]
- MacLaren RE, Pearson RA, MacNeil A, Douglas RH, Salt TE, Akimoto M, Swaroop A, Sowden JC, Ali RR. Retinal repair by transplantation of photoreceptor precursors. *Nature* 2006;444:203–207. [PubMed: 17093405]
- Makowski A, Brzostek S, Cohen RN, Hollenberg AN. Determination of nuclear receptor corepressor interactions with the thyroid hormone receptor. *Mol Endocrinol* 2003;17:273–286. [PubMed: 12554754]
- Mali RS, Zhang X, Hoerauf W, Doyle D, Devitt J, Loffreda-Wren J, Mitton KP. FIZ1 is expressed during photoreceptor maturation, and synergizes with NRL and CRX at rod-specific promoters in vitro. *Exp Eye Res* 2007;84:349–360. [PubMed: 17141759]
- Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. *Cell* 1995;83:841–850. [PubMed: 8521508]
- Martinez E, Palhan VB, Tjernberg A, Lymar ES, Gamper AM, Kundu TK, Chait BT, Roeder RG. Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors in vivo. *Mol Cell Biol* 2001;21:6782–6795. [PubMed: 11564863]
- Martinez-Gimeno M, Maseras M, Baiget M, Beneito M, Antinolo G, Ayuso C, Carballo M. Mutations P51U and G122E in retinal transcription factor NRL associated with autosomal dominant and sporadic retinitis pigmentosa. *Hum Mutat* 2001;17:520. [PubMed: 11385710]
- Martinez-Morales JR, Signore M, Acampora D, Simeone A, Bovolenta P. Otx genes are required for tissue specification in the developing eye. *Development* 2001;128:2019–2030. [PubMed: 11493524]
- Martinez-Morales JR, Dolez V, Rodrigo I, Zaccarini R, Leconte L, Bovolenta P, Saule S. OTX2 activates the molecular network underlying retina pigment epithelium differentiation. *J Biol Chem* 2003;278:21721–21731. [PubMed: 12663655]
- Matsuo I, Kuratani S, Kimura C, Takeda N, Aizawa S. Mouse Otx2 functions in the formation and patterning of rostral head. *Genes Dev* 1995;9:2646–2658. [PubMed: 7590242]
- Mears AJ, Kondo M, Swain PK, Takada Y, Bush RA, Saunders TL, Sieving PA, Swaroop A. *Nrl* is required for rod photoreceptor development. *Nat Genet* 2001;29:447–452. [PubMed: 11694879]
- Milam AH, Rose L, Cideciyan AV, Barakat MR, Tang WX, Gupta N, Aleman TS, Wright AF, Stone EM, Sheffield VC, Jacobson SG. The nuclear receptor NR2E3 plays a role in human retinal photoreceptor differentiation and degeneration. *Proc Natl Acad Sci U S A* 2002;99:473–478. [PubMed: 11773633]
- Mitton KP, Swain PK, Chen S, Xu S, Zack DJ, Swaroop A. The leucine zipper of NRL interacts with the CRX homeodomain. A possible mechanism of transcriptional synergy in rhodopsin regulation. *J Biol Chem* 2000;275:29794–29799. [PubMed: 10887186]
- Mitton KP, Swain PK, Khanna H, Dowd M, Apel IJ, Swaroop A. Interaction of retinal bZIP transcription factor NRL with Flt3-interacting zinc-finger protein Fiz1: possible role of Fiz1 as a transcriptional repressor. *Hum Mol Genet* 2003;12:365–373. [PubMed: 12566383]
- Mori M, Ghyselinck NB, Chambon P, Mark M. Systematic immunolocalization of retinoid receptors in developing and adult mouse eyes. *Invest Ophthalmol Vis Sci* 2001;42:1312–1318. [PubMed: 11328745]
- Morrow EM, Furukawa T, Lee JE, Cepko CL. NeuroD regulates multiple functions in the developing neural retina in rodent. *Development* 1999;126:23–36. [PubMed: 9834183]
- Ng L, Hurley JB, Dierks B, Srinivas M, Salto C, Vennstrom B, Reh TA, Forrest D. A thyroid hormone receptor that is required for the development of green cone photoreceptors. *Nat Genet* 2001;27:94–98. [PubMed: 11138006]
- Nikonov SS, Kholodenko R, Lem J, Pugh EN Jr. Physiological features of the S- and M-cone photoreceptors of wild-type mice from single-cell recordings. *J Gen Physiol* 2006;127:359–374. [PubMed: 16567464]

- Nikonov SS, Daniele LL, Zhu X, Craft CM, Swaroop A, Pugh EN Jr. Photoreceptors of *Nrl* $-/-$ mice coexpress functional S- and M-cone opsins having distinct inactivation mechanisms. *J Gen Physiol* 2005;125:287–304. [PubMed: 15738050]
- Nishida A. [Mechanisms of retinal photoreceptor cell fate determination]. *Nippon Ganka Gakkai Zasshi* 2005;109:708–716. [PubMed: 16363664]
- Nishida A, Furukawa A, Koike C, Tano Y, Aizawa S, Matsuo I, Furukawa T. *Otx2* homeobox gene controls retinal photoreceptor cell fate and pineal gland development. *Nat Neurosci* 2003;6:1255–1263. [PubMed: 14625556]
- Nishiguchi KM, Friedman JS, Sandberg MA, Swaroop A, Berson EL, Dryja TP. Recessive *NRL* mutations in patients with clumped pigmentary retinal degeneration and relative preservation of blue cone function. *Proc Natl Acad Sci U S A* 2004;101:17819–17824. [PubMed: 15591106]
- Oh EC, Khan N, Novelli E, Khanna H, Strettoi E, Swaroop A. From the Cover: Transformation of cone precursors to functional rod photoreceptors by *bZIP* transcription factor *NRL*. *Proc Natl Acad Sci U S A* 2007;104:1679–1684. [PubMed: 17242361]
- Olsson JE, Gordon JW, Pawlyk BS, Roof D, Hayes A, Molday RS, Mukai S, Cowley GS, Berson EL, Dryja TP. Transgenic mice with a rhodopsin mutation (*Pro23His*): a mouse model of autosomal dominant retinitis pigmentosa. *Neuron* 1992;9:815–830. [PubMed: 1418997]
- Palhan VB, Chen S, Peng GH, Tjernberg A, Gamper AM, Fan Y, Chait BT, La Spada AR, Roeder RG. Polyglutamine-expanded ataxin-7 inhibits *STAGA* histone acetyltransferase activity to produce retinal degeneration. *Proc Natl Acad Sci U S A* 2005;102:8472–8477. [PubMed: 15932940]
- Peichl L. Diversity of mammalian photoreceptor properties: adaptations to habitat and lifestyle? *Anat Rec A Discov Mol Cell Evol Biol* 2005;287:1001–1012. [PubMed: 16200646]
- Peng GH, Chen S. Chromatin immunoprecipitation identifies photoreceptor transcription factor targets in mouse models of retinal degeneration: new findings and challenges. *Vis Neurosci* 2005;22:575–586. [PubMed: 16332268]
- Peng GH, Ahmad O, Ahmad F, Liu J, Chen S. The photoreceptor-specific nuclear receptor *Nr2e3* interacts with *Crx* and exerts opposing effects on the transcription of rod versus cone genes. *Hum Mol Genet* 2005;14:747–764. [PubMed: 15689355]
- Pennesi ME, Cho JH, Yang Z, Wu SH, Zhang J, Wu SM, Tsai MJ. *BETA2/NeuroD1* null mice: a new model for transcription factor-dependent photoreceptor degeneration. *J Neurosci* 2003;23:453–461. [PubMed: 12533605]
- Plouhinec JL, Sauka-Spengler T, Germot A, Le Mentec C, Cabana T, Harrison G, Pieau C, Sire JY, Veron G, Mazan S. The mammalian *Crx* genes are highly divergent representatives of the *Otx5* gene family, a gnathostome orthology class of orthodenticle-related homeogenes involved in the differentiation of retinal photoreceptors and circadian entrainment. *Mol Biol Evol* 2003;20:513–521. [PubMed: 12654938]
- Ragge NK, Brown AG, Poloschek CM, Lorenz B, Henderson RA, Clarke MP, Russell-Eggitt I, Fielder A, Gerrelli D, Martinez-Barbera JP, Ruddle P, Hurst J, Collin JR, Salt A, Cooper ST, Thompson PJ, Sisodiya SM, Williamson KA, Fitzpatrick DR, van Heyningen V, Hanson IM. Heterozygous mutations of *OTX2* cause severe ocular malformations. *Am J Hum Genet* 2005;76:1008–1022. [PubMed: 15846561]
- Rehemtulla A, Warwar R, Kumar R, Ji X, Zack DJ, Swaroop A. The basic motif-leucine zipper transcription factor *Nrl* can positively regulate rhodopsin gene expression. *Proc Natl Acad Sci U S A* 1996;93:191–195. [PubMed: 8552602]
- Rivolta C, Berson EL, Dryja TP. Dominant Leber congenital amaurosis, cone-rod degeneration, and retinitis pigmentosa caused by mutant versions of the transcription factor *CRX*. *Hum Mutat* 2001a;18:488–498. [PubMed: 11748842]
- Rivolta C, Peck NE, Fulton AB, Fishman GA, Berson EL, Dryja TP. Novel frameshift mutations in *CRX* associated with Leber congenital amaurosis. *Hum Mutat* 2001b;18:550–551. [PubMed: 11748859]
- Roberts MR, Hendrickson A, McGuire CR, Reh TA. Retinoid X receptor (γ) is necessary to establish the S-opsin gradient in cone photoreceptors of the developing mouse retina. *Invest Ophthalmol Vis Sci* 2005;46:2897–2904. [PubMed: 16043864]

- Roberts MR, Srinivas M, Forrest D, Morreale de Escobar G, Reh TA. Making the gradient: thyroid hormone regulates cone opsin expression in the developing mouse retina. *Proc Natl Acad Sci U S A* 2006;103:6218–6223. [PubMed: 16606843]
- Rodieck, RW. *The First Steps in Seeing*. 1 Edition.. Sinauer Associates, Inc.; Sunderland, MA: 1998.
- Rohlich P, van Veen T, Szel A. Two different visual pigments in one retinal cone cell. *Neuron* 1994;13:1159–1166. [PubMed: 7946352]
- Scheetz TE, Kim KY, Swiderski RE, Philp AR, Braun TA, Knudtson KL, Dorrance AM, DiBona GF, Huang J, Casavant TL, Sheffield VC, Stone EM. Regulation of gene expression in the mammalian eye and its relevance to eye disease. *Proc Natl Acad Sci U S A* 2006;103:14429–14434. [PubMed: 16983098]
- Shibusawa N, Hashimoto K, Nikrodhanond AA, Liberman MC, Applebury ML, Liao XH, Robbins JT, Refetoff S, Cohen RN, Wondisford FE. Thyroid hormone action in the absence of thyroid hormone receptor DNA-binding in vivo. *J Clin Invest* 2003;112:588–597. [PubMed: 12925699]
- Sohocki MM, Sullivan LS, Mintz-Hittner HA, Birch D, Heckenlively JR, Freund CL, McInnes RR, Daiger SP. A range of clinical phenotypes associated with mutations in CRX, a photoreceptor transcription-factor gene. *Am J Hum Genet* 1998;63:1307–1315. [PubMed: 9792858]
- Srinivas M, Ng L, Liu H, Jia L, Forrest D. Activation of the blue opsin gene in cone photoreceptor development by retinoid-related orphan receptor beta. *Mol Endocrinol* 2006;20:1728–1741. [PubMed: 16574740]
- Strettoi E, Mears AJ, Swaroop A. Recruitment of the rod pathway by cones in the absence of rods. *J Neurosci* 2004;24:7576–7582. [PubMed: 15329405]
- Swain PK, Hicks D, Mears AJ, Apel IJ, Smith JE, John SK, Hendrickson A, Milam AH, Swaroop A. Multiple phosphorylated isoforms of NRL are expressed in rod photoreceptors. *J Biol Chem* 2001;276:36824–36830. [PubMed: 11477108]
- Swaroop A, Xu I, Pawar H, Jackson A, Skolnick C. A conserved retina-specific gene encodes a basic motif/leucine zipper domain. *Proc Natl Acad Sci U S A* 1992;89:266–270. [PubMed: 1729696]
- Szanto A, Narkar V, Shen Q, Uray IP, Davies PJ, Nagy L. Retinoid X receptors: X-ploring their (patho) physiological functions. *Cell Death Differ* 11 Suppl 2004;2:S126–143.
- Tahayato A, Sonnevile R, Pichaud F, Wernet MF, Papatsenko D, Beaufils P, Cook T, Desplan C. Otd/Crx, a dual regulator for the specification of ommatidia subtypes in the *Drosophila* retina. *Dev Cell* 2003;5:391–402. [PubMed: 12967559]
- Takezawa S, Yokoyama A, Okada M, Fujiki R, Iriyama A, Yanagi Y, Ito H, Takada I, Kishimoto M, Miyajima A, Takeyama KI, Umesono K, Kitagawa H, Kato S. A cell cycle-dependent co-repressor mediates photoreceptor cell-specific nuclear receptor function. *Embo J*. 2007
- Treisman J, Gonczy P, Vashishtha M, Harris E, Desplan C. A single amino acid can determine the DNA binding specificity of homeodomain proteins. *Cell* 1989;59:553–562. [PubMed: 2572327]
- Turner DL, Cepko CL. A common progenitor for neurons and glia persists in rat retina late in development. *Nature* 1987;328:131–136. [PubMed: 3600789]
- Ueno S, Kondo M, Miyata K, Hirai T, Miyata T, Usukura J, Nishizawa Y, Miyake Y. Physiological function of S-cone system is not enhanced in rd7 mice. *Exp Eye Res* 2005;81:751–758. [PubMed: 16005871]
- Vandendries ER, Johnson D, Reinke R. orthodenticle is required for photoreceptor cell development in the *Drosophila* eye. *Dev Biol* 1996;173:243–255. [PubMed: 8575625]
- von Schantz M, Lucas RJ, Foster RG. Circadian oscillation of photopigment transcript levels in the mouse retina. *Brain Res Mol Brain Res* 1999;72:108–114. [PubMed: 10521605]
- Wang QL, Chen S, Esumi N, Swain PK, Haines HS, Peng G, Melia BM, McIntosh I, Heckenlively JR, Jacobson SG, Stone EM, Swaroop A, Zack DJ. QRX, a novel homeobox gene, modulates photoreceptor gene expression. *Hum Mol Genet* 2004;13:1025–1040. [PubMed: 15028672]
- Wang X, Xu S, Rivolta C, Li LY, Peng GH, Swain PK, Sung CH, Swaroop A, Berson EL, Dryja TP, Chen S. Barrier to autointegration factor interacts with the cone-rod homeobox and represses its transactivation function. *J Biol Chem* 2002;277:43288–43300. [PubMed: 12215455]
- Watanabe T, Raff MC. Rod photoreceptor development in vitro: intrinsic properties of proliferating neuroepithelial cells change as development proceeds in the rat retina. *Neuron* 1990;4:461–467. [PubMed: 2138470]

- Wetts R, Fraser SE. Multipotent precursors can give rise to all major cell types of the frog retina. *Science* 1988;239:1142–1145. [PubMed: 2449732]
- Wright AF, Reddick AC, Schwartz SB, Ferguson JS, Aleman TS, Kellner U, Jurkles B, Schuster A, Zrenner E, Wissinger B, Lennon A, Shu X, Cideciyan AV, Stone EM, Jacobson SG, Swaroop A. Mutation analysis of NR2E3 and NRL genes in Enhanced S Cone Syndrome. *Hum Mutat* 2004;24:439. [PubMed: 15459973]
- Yan RT, Ma W, Liang L, Wang SZ. bHLH genes and retinal cell fate specification. *Mol Neurobiol* 2005;32:157–171. [PubMed: 16215280]
- Yanagi Y, Takezawa S, Kato S. Distinct functions of photoreceptor cell-specific nuclear receptor, thyroid hormone receptor beta2 and CRX in one photoreceptor development. *Invest Ophthalmol Vis Sci* 2002;43:3489–3494. [PubMed: 12407160]
- Yanagi Y, Masuhiro Y, Mori M, Yanagisawa J, Kato S. p300/CBP acts as a coactivator of the cone-rod homeobox transcription factor. *Biochem Biophys Res Commun* 2000;269:410–414. [PubMed: 10708567]
- Yen PM, Sunday ME, Darling DS, Chin WW. Isoform-specific thyroid hormone receptor antibodies detect multiple thyroid hormone receptors in rat and human pituitaries. *Endocrinology* 1992;130:1539–1546. [PubMed: 1537303]
- Yin L, Wang J, Klein PS, Lazar MA. Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock. *Science* 2006;311:1002–1005. [PubMed: 16484495]
- Yoshida S, Mears AJ, Friedman JS, Carter T, He S, Oh E, Jing Y, Farjo R, Fleury G, Barlow C, Hero AO, Swaroop A. Expression profiling of the developing and mature *Nrl*^{-/-} mouse retina: identification of retinal disease candidates and transcriptional regulatory targets of *Nrl*. *Hum Mol Genet* 2004;13:1487–1503. [PubMed: 15163632]
- Young RW. Cell differentiation in the retina of the mouse. *Anat Rec* 1985;212:199–205. [PubMed: 3842042]
- Yu J, He S, Friedman JS, Akimoto M, Ghosh D, Mears AJ, Hicks D, Swaroop A. Altered Expression of Genes of the Bmp/Smad and Wnt/Calcium Signaling Pathways in the Cone-only *Nrl*^{-/-} Mouse Retina, Revealed by Gene Profiling Using Custom cDNA Microarrays. *J Biol Chem* 2004;279:42211–42220. [PubMed: 15292180]
- Yu X, Lin J, Zack DJ, Qian J. Computational analysis of tissue-specific combinatorial gene regulation: predicting interaction between transcription factors in human tissues. *Nucleic Acids Res* 2006;34:4925–4936. [PubMed: 16982645]
- Zhang CL, Zou Y, Yu RT, Gage FH, Evans RM. Nuclear receptor TLX prevents retinal dystrophy and recruits the corepressor atrophin1. *Genes Dev* 2006;20:1308–1320. [PubMed: 16702404]

Figure 1. Schematic diagram of photoreceptor-specific transcription factors

The domain structures of photoreceptor-specific transcription factors discussed in this paper are presented in scale. Conserved domains for classifying families of transcription factors are indicated in black, other regions of homology conserved among different family members are indicated by stippling. Functional regions are indicated above the box representing the factor; sites of mutations discussed in the text are indicated by arrowheads below the box. N- and C-terminals are indicated, and the number below the C-terminal end indicates the number of amino acids in the human protein. HOMEO, homeodomain; b, basic domain; L Zipper, leucine zipper domain; Zn F, zinc finger domain.

Mouse

Human

Figure 2. Distribution of cones and opsin expression in mouse and human retina

A. In the mouse, cones are scattered throughout the retina, with M-cones (green) predominating in the dorsal retina and S-cones (blue) predominating in the ventral retina. Many cones express both opsins. B. M-opsin and S-opsin are expressed in complementary dorsal (D) to ventral (V) gradients across the mouse retina, likely in response to a gradient of thyroid hormone (TH), which is established between P4 and P10, and is highest in the dorsal retina as indicated. C. This graph shows the spatial density of rods (blue) and cones (orange) in a horizontal strip of human retina across the fovea (centered at position 0) and optic disc. Cones are concentrated in the fovea, and rods are excluded from this region. Elsewhere in the retina, rods predominate and cones are sparse. D. Within the fovea, cones expressing either green or red opsin predominate, with cones expression blue opsin found sparsely around the peripheral fovea region. Human cones only express a single type of opsin.

Panels A and B are from (Applebury et al., 2000), reprinted by permission from Cell Press, with TH gradient added from (Roberts et al., 2006). Panel C is from (Rodieck, 1998), pg. 43,

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Figure 3. ChIP analysis demonstrating that network transcription factors bind to their own and each other's promoters

Antibodies against Crx, Nrl, Nr2e3, Trβ2, and NeuroD1 were used to immunoprecipitate the bound chromatin fragments from wild-type (WT), *Crx*^{-/-}, *Nrl*^{-/-}, or *Nr2e3*^{rd7/rd7} (“Nr2e3^{-/-}”) retinæ. Primers specific to the promoter regions of the genes listed on the left [(Peng and Chen, 2005); Table 3] were used to detect the presence of the candidate promoter regions in the immunoprecipitates by PCR. A band indicates that the transcription factor recognized by the immunoprecipitating antibody is bound to the promoter region of the indicated regulator or target gene. Target genes examined include *S-opsin* (*Sop*), *M-opsin* (*Mop*), *rhodopsin* (*Rho*), and interphotoreceptor binding protein (*Rbp3*), all of which are expressed in photoreceptors. *GluR6*, which is expressed in bipolar cells but not photoreceptors, serves as a control for photoreceptor specificity. In addition, PCR reactions using primers against DNA sequences immediately 3' of each gene gave no bands (data not shown), confirming regulatory region-specific binding. Control immunoprecipitates using purified non-specific rabbit or goat IgG yielded no specific promoter sequences from WT (second lanes from the right) or knockout (data not shown) mice. Samples of retina homogenates (“input”) from WT (far right lanes) and knockout (data not shown) mice serve as positive controls for PCR.

Figure 4. Model for transcription factor network regulation of photoreceptor subtype development
 Photoreceptor subtypes develop from photoreceptor precursors derived from multi-potent progenitors via three major pathways (thick arrows). Photoreceptor transcription factors that play a major role in this process are listed based on their epistatic relationship as determined by in vivo and/or in vitro functional studies. Thin lines show protein-promoter interactions; solid lines show interactions reported here and/or previously; dotted lines are from unpublished data. Arrows indicate positive regulation, while blocked lines represent inhibition/suppression. Absence of lines indicates that the relationship remains to be determined.

Table 1

Transcription regulators for photoreceptor gene expression, development and/or maintenance

Factors	Subtype	Expression in photoreceptors Period ¹	Function ² / targets ³	Key References
Photoreceptor-enriched				
Homeodomain				
Otx2	dev ph precursors	E10.5-P6	act / <i>Crx</i> , <i>Rbp3</i> , etc.	Nishida et al., 2003
Crx	dev/ad rods/cones	E12.5-ad	act / <i>opsins</i> , reg. factors	Chen et al., 1997; Furukawa et al., 1997; 1999
Rax	dev/ad ph	P10.5-ad	act / <i>Rho</i> , <i>Arr</i> , <i>Rbp3</i>	Zhang et al., 2000; Kimura et al., 2000
Qrx/RaxL	dev/ad ph	ad(Bo/Hu/Ch/Xe)	act / <i>Rho</i>	Chen et al., 2002; Wang et al., 2004
bZIP				
Nrl	dev/ad rods	E13.5-ad	act / <i>Rho</i> , <i>Nr2e3</i> , <i>Pde6b</i> , etc.	Liu et al., 1996; Mears et al., 2001
bHLH				
NeuroD1	dev rods	E12.5-P3	ph survival/unclear	Morrow, et al., 1999, Pennesi et al., 2003
Mash1	dev rods	E13.5-P3	act / <i>Rho</i>	Guillemot et al., 1993; Ahmad et al., 1995
Math5	dev precursors	E11.5-birth	rep / <i>NeuroD1</i> , <i>Neurog2</i>	Brown, et al, 1998; Le et al., 2006
Neurog2	dev precursors	E10-birth	act? / <i>NeuroD1</i>	Ma and Wang, 2006; Yan et al., 2005
Nuclear Receptors				
Trβ2	dev cones	E16-ad	dual / <i>S-</i> and <i>M-opsin</i>	Yanagi et al., 2002; Roberts et al., 2006
Rxry	dev cones	E 14.5-ad	rep / <i>S-opsin</i>	Roberts et al., 2005
Rorβ	dev cones	E12.5-ad	act / <i>S-opsin</i>	Chow et al., 1998; Srinivas et al., 2006
Nr1d1(Rev-erb-α)	dev ph	P0-ad	dual? / circadian genes	Cheng et al., 2004
Nr2e1(Tlx)	dev cones	turned on E8	dual / <i>S-opsin</i> , <i>Pax2</i> , <i>Rar</i>	Zhang et al., 2006
Nr2e3	dev/ad rods	E18-ad	dual / all <i>opsins</i>	Kobayashi et al., 1999; Peng et al., 2005
Ubiquitously-expressed				
Rb1	rods	P12-P21	act? / <i>Nrl</i>	Zhang et al., 2004
Sp1, Sp4	dev/ad ph	E12.5-ad	act / <i>Pde6b</i> , <i>Rho</i>	Lerner et al., 2001
ataxin-7	dev/ad ph	E12.5-ad	coact / <i>opsins</i>	Palhan et al., 2005
Gcn5	dev/ad ph	E12.5-ad	coact / <i>opsins</i>	Palhan et al., 2005
Cbp/p300	dev/ad ph	E12.5-ad	coact / <i>opsins</i>	Yanagi et al., 2000

Abbreviations: act-activator; ad-adult; coact-co-activator; dev-developmental; dual-activator and repressor; ph-photoreceptors; reg.-regulatory; rep-repressor; *Arr*-rod arrestin; *Neurog2*-neurogenin 2.

¹ In mice or in species as noted: Bo-bovine, Hu-human, Ch-chicken, Xe-Xenopus.

² Based on loss-of-function studies.

³ direct targets if known (based on protein-DNA binding assays).

Table 2
P14 retinal mRNA levels relative to WT (qRT-PCR analysis)

Genes	<i>Crx</i> ^{-/-}	Mouse strains <i>Nrl</i> ^{-/-}	<i>Nr2e3</i> ^{-/-}
Regulators			
<i>Otx2</i>	2.50 ± 0.12 *	1.00 ± 0.12	1.00 ± 0.12
<i>Crx</i>	0.05 ± 0.06 *	1.00 ± 0.06	1.03 ± 0.06
<i>Nrl</i>	0.67 ± 0.06 *	0.06 ± 0.06 *	1.00 ± 0.06
<i>Nr2e3</i>	0.61 ± 0.05 *	0.04 ± 0.15 *	0.04 ± 0.17 *
<i>Trβ2</i>	0.46 ± 0.15 *	1.02 ± 0.15	1.02 ± 0.17
<i>Rxry</i>	4.32 ± 0.10 *	1.57 ± 0.15 *	1.32 ± 0.10 *
<i>Rorβ</i>	2.22 ± 0.15 *	1.78 ± 0.06 *	1.28 ± 0.10 *
<i>NeuroD1</i>	0.75 ± 0.15 *	1.01 ± 0.15	1.00 ± 0.15
Targets			
<i>Sop</i>	0.09 ± 0.06 *	2.14 ± 0.10 *	1.23 ± 0.10 *
<i>Mop</i>	0.06 ± 0.06 *	1.17 ± 0.06 *	1.19 ± 0.10 *
<i>Rho</i>	0.12 ± 0.06 *	0.07 ± 0.06 *	0.84 ± 0.06 *
<i>Rbp3</i>	1.00 ± 0.10	1.00 ± 0.15	1.00 ± 0.15

Results of quantitative RT-PCR are presented as the ratio of the knockout to wild-type expression level of each gene (see Experimental Procedures for calculations). The numbers represent the mean value ± standard deviation from three repeats.

* $P < 0.05$ based on paired student t-test.

Table 3

PCR primers for ChIP and quantitative RT-PCR

Genes	Assays	Sense	Anti-sense
<i>Trβ2</i> :	ChIP (-321/-53) ¹	5'-ACCTGCCTGCCATTTTCCC-3'	5'-ATTTGCCAGCCCCCTGAAC-3'
	qRT-PCR ²	5'-GCACATCTCCCTGAAGAAAAGC-3'	5'-TCCCCACACACTACACAGAGC-3'
<i>NeuroD1</i> :	ChIP (-1504/-1336)	5'-TCCAGCCACTCAACCCTGAC-3'	5'-GAGGAGGAGGAGGAATGGTG-3'
<i>Otx2</i> :	qRT-PCR	5'-CGCTCAGCATCAGCAACTC-3'	5'-CTTGTCTGCCTCGTGTTC-3'
<i>Crx</i> :	qRT-PCR	5'-ACTTGCCAGAATCCAGGGTG-3'	5'-TGAGCCAGCATAGCCTTGAC-3'
<i>Nrl</i> :	qRT-PCR	5'-TGTCCTACTCAAGTGCCC-3'	5'-TGCTGTTTCTGCTGCTGTCG-3'
<i>Nr2e3</i> :	qRT-PCR	5'-TTCTGGTTCTGACAGTGAACG-3'	5'-AAGGCTCCCGCTTATTTC-3'
<i>Rvrγ</i> :	ChIP (-362/-109)	5'-AGTCCCAGGTGATGCTAAGC-3'	5'-TTCTAAGATGTGCTGCCCC-3'
	qRT-PCR	5'-AAAGGGCTCTGTCTCTCTTG-3'	5'-CGGGTGGCACAATCTATTAGC-3'
<i>Rorβ</i> :	ChIP (-471/-96)	5'-CAATGCTCTTGGCTCTCCG-3'	5'-ATCTTTGTTATCCCGACAGGTG-3'
	qRT-PCR ²	5'-AAAGAGACAGAGGAGAGAGGGG-3'	5'-CAGTTAGAGGATGCTGGGTGC-3'
		5'-AAGGGATTCTTCAGGAGGAGC-3'	5'-CCGCTGCTTCTTGGACATC-3'

¹ For ChIP primers, the numbers in parentheses indicate the position relative to the transcription start site as +1.

² Note that qRT-PCR primers for *Trβ2* recognize only the β2 isoform, while *Rorβ* primers recognize both β1 and β2 isoforms.