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Characterization of transgenic mouse lines expressing Cre-recombinase in the retina

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Abstract

The mammalian retina consists of five major classes of neuronal cells, as well as glial cells, and it contains more than 50 cell types. The ability to manipulate gene expression in specific cell type(s) in the retina is important for understanding the molecular mechanisms of retinal function and diseases. The Cre/*loxP* recombination system has been shown to be a powerful tool, allowing gene deletion, over-expression, and ectopic expression *in vivo* in a cell- and tissue-specific fashion. The key to this tool is the availability of Cre mouse lines with cell- or tissue-type specific expression of Cre recombinase. To date, a large number of Cre-transgenic mouse lines have been generated to target Cre recombinase expression to specific neuronal and glial cell populations in the CNS; however, information about the expression patterns of Cre recombinase lines in the retina is largely lacking. In this study, we examined and characterized the expression patterns of Cre recombinase in the retinas of 15 Cre-transgenic mouse lines. Significant Cre-induced recombination or expression of Cre recombinase was observed in the majority of these lines. In particular, we found several Cre lines in which the Cre-induced recombination was found to target exclusively or predominantly a single type or class of retinal cells, including bistratified retinal ganglion cells, starburst amacrine cells, rod bipolar cells, and Müller glial cells. In other lines, the Cre-induced recombination was found in several retinal cell types. These Cre lines provide a valuable resource for retinal research.

Keywords

Cre-transgenic mice; green fluorescent protein; retina; bipolar cell; amacrine cell; ganglion cell

Introduction

The retina, the first site of visual processing, is part of the central nervous system (CNS) and has a complex, layered structure. The mammalian retina consists of five major classes of neuronal cells: photoreceptors, bipolar cells, horizontal cells, amacrine cells, and ganglion cells. Each neuronal cell class is composed of multiple types, and over 50 neuronal cell types are believed to be present in the mammalian retina (Masland, 2001; Wässle, 2004). The retina also contains several types of glial cells, predominantly Müller glial cells. The ability to manipulate gene expression in specific cell type(s) in the retina is important for understanding the molecular mechanisms of retinal function and diseases.

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Genetically modified mice have been an invaluable tool for genetic manipulation in an animal model *in vivo*. In particular, the Cre/*loxP* recombination system has been shown to be a powerful tool, allowing gene deletion, over-expression, and ectopic expression *in vivo* in a cell- and tissue-specific fashion (Nagy, 2000; Branda et al., 2004). The key to this tool is the availability of Cre mouse lines with cell- or tissue-specific expression of Cre recombinase. Although a number of Cre mouse lines have been generated to express Cre recombinase in specific retinal cells (Barski et al., 2000; Akimoto et al., 2004; Rowan and Cepko, 2004; Li et al., 2005; Le et al., 2004; 2006; Zhang et al., 2005; Saito et al., 2005; Jimeno et al., 2006), most of these lines were targeted to photoreceptors or bipolar cells. In addition, a large number of Cre-transgenic mouse lines have been generated to target expression of Cre recombinase to specific neuronal and glial cell populations in the CNS (Gong et al., 2007; Gavériaux-Ruff and Kieffer, 2007; also see The Jackson Laboratory at <http://jaxmice.jax.org/research/cre/strainlist.html>; Mutant Mouse Regional Resource Center at <http://www.mmrrc.org>). Information regarding the expression patterns of Cre recombinase in the retina, however, is largely lacking. In this study, we examined and characterized the expression patterns of Cre recombinase in the retinas of 15 Cre-transgenic mouse lines.

Experimental procedures

Animals

The Cre lines were chosen mainly based on their availability and known expression in neuronal or glial cells in the CNS. Except for the Tg(Chat-cre)24Gsat which was obtained from Mutant Mouse Regional Resource Center (MMRRC; University of California, Davis, CA, USA), all other lines were obtained from the Jackson Laboratory (Bar Harbor, ME, USA): 1. C57BL/6-Tg(Grik4-cre)G32-4Stl/J (Grik4-cre), produced by driving the Cre recombinase gene under the control of a bacterial artificial chromosome (BAC) clone containing the mouse KA-1 (one of kainate receptor subunits) genomic sequence (Nakazawa et al., 2002); 2. B6.129S6-Chat^{tm1(cre)Lowl}/J (ChAT-cre/Jax), produced by knocking in the Cre recombinase gene downstream of the endogenous ChAT gene (see the description of the Jackson Laboratory); 3. Tg(Chat-cre)24Gsat (ChAT-cre/Gsat), produced by driving the Cre recombinase gene under the control of a BAC clone containing the ChAT genomic sequence (Gong et al., 2007); 4. Tg(Pcp2-cre)1Amc/J (Pcp2-cre/GFP), generated by driving Cre recombinase and GFP or a GFP derivative under the control of a modified mouse Purkinje cell protein 2 (Pcp2) promoter enhancer (Lewis et al., 2004; also see the description of the Jackson Laboratory); 5. Tg(Pcp2-cre)2Mpin/J, generated by inserting the Cre recombinase gene into exon 4 of the Purkinje cell protein 2 (Pcp2) gene (Barski et al., 2000); 6. Tg(Chx10-EGFP/cre-ALPP)2Clc/J (Chx10-cre/GFP), produced by an EGFP/cre fusion transgene under the control of a BAC clone containing the mouse transcription factor Chx10 (*C. elegans* *ceh-10* homeo domain containing homolog) promoter (Rowan and Cepko, 2004); 7. B6.129P2(Cg)-Foxg1^{tm1(cre)Skm}/J (Foxg1-cre), generated by replacing Forkhead box G1 (Foxg1; a winged-helix transcription factor) coding sequences with the Cre recombinase gene (Hebert et al., 2000); 8. B6.Cg-Tg(Nes-cre)1Kln/J (Nes-cre), produced by driving the Cre recombinase gene under the control of the promoter/enhancer of the second intron of the rat nestin (Nes) gene (Zimmerman et al., 1994; Tronche et al., 1999); 9. Tg(Eno2-cre)39Jme/J (Eno2-cre), generated by driving Cre recombinase gene under the control of the rat neuron-specific enolase (Eno2) gene promoter (Frugier et al., 2000; Forss-Petter, 1999); 10. FVB/N-Tg(Thy1-cre)1Vln/J (Thy1-cre), generated by driving the Cre recombinase gene under the control of the mouse thymocyte differentiation antigen 1 (Thy-1) gene promoter (Dewachter et al., 2002); 11. TgN(Wnt1-GAL4)11Rth TgN(Wnt1-Cre)11Rth (Wnt1-cre), generated by driving the Cre recombinase gene under the control of the wingless type mouse mammary tumor virus integration site gene family member 1 (Wnt1) promoter/enhancer (Danielian et al., 1998); 12. B6.129S1-Mnx1^{tm4(cre)Tmj}/J (Mnx1-cre), generated by replacing a portion of the first exon of the motor neuron and pancreas homeobox

1 (MNX1; also known as Homeobox HB9) gene with the Cre recombinase gene (Arber et al., 1999); 13. B6.Cg-Tg(Camk2a-cre)T29-1Stl/J (Camk2a-cre), generated by driving the Cre recombinase gene under the control of an 8.5 Kb sequence encoding the mouse calcium/calmodulin-dependent protein kinase II alpha (Camk2a) promoter (Tsien et al., 1996); 14. B6.129S2-Emx1^{tm1(cre)Krl}/J (Emx1-cre), generated by inserting an IRES-cre gene into the exon encoding the 3' untranslated region of the mouse homeobox gene Emx1 (a mouse homologue of *Drosophila* empty homolog 1) (Gorski et al., 2002); 15. FVB-Tg(Nr5a1-cre)2Lowl/J (Nr5a1-cre), produced by driving the Cre recombinase gene under the control of a BAC clone containing steroidogenic factor-1 regulatory elements (Dhillon et al., 2006).

Since the Pcp2-cre/GFP and Chx10-cre/GFP lines already carry GFP within the Cre transgene construct, the expression of Cre recombinase in these two lines was examined by GFP and/or immunolabeling with an antibody against Cre. For all other Cre lines, the expression pattern of the Cre recombinase was evaluated by crossing them with a ROSA26-EYFP Cre excision reporter strain (homozygote): B6.129X1-Gt(ROSA)26Sor^{tm1(EYFP)Cos}/J, obtained from the Jackson Laboratory (Srinivas et al., 2001). A potential advantage of examining the GFP expression with Cre/LoxP recombination is that it could also provide the information about the efficiency of Cre/LoxP recombination. The offspring were genotyped for the presence of Cre recombinase by PCR using DNA prepared from tail biopsies and the following primer pairs: ACCAGCCAGCTATCAACTCG and TTACATTGGTCCAGCCACC. The expression of fluorescent protein expression was evaluated in the Cre-positive offspring at one month of age. All animal handling procedures were approved by the Institutional Animal Care and Use Committee at Wayne State University and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Immunocytochemical staining

Mice were deeply anesthetized with CO₂ and decapitated. The retinas were fixed in the eyecups with 4% paraformaldehyde in 0.1 M phosphate buffer (PB) for 20 minutes. The expression of EYFP in the retina was examined in retinal whole-mounts and vertical sections. For whole-mounts, after fixation the retina was dissected free in PB solution, flat mounted on slides, and cover slipped. For retinal vertical sections, the retinas were cryoprotected in a sucrose gradient (10%, 20%, and 30% w/v in PB), and cryostat sections were cut at 20 µm.

The EYFP or GFP fluorescence without enhancement with antibody was sufficient to visualize the EYFP- or GFP-expressing cells. In order to enhance the signal and reveal the fine cell processes, specimens were stained with antibodies against EYFP or GFP to generate all of the fluorescence images shown in this study. The following antibodies were used in this study: rabbit anti-GFP (1:2000; Cat # A21311; Molecular Probes), mouse anti-GFP (1:2000; Cat # 75-132, clone N86/38; Neuromab), goat anti-choline acetyl transferase (ChAT, 1:2000; Cat # AB144P; Chemicon), rabbit anti-Goalpha (1:30000; Cat # 07-634; Millipore), rabbit anti-glutamine synthetase (GS, 1:10000; Cat # G2781; Sigma), mouse anti-protein kinase Cα (PKC, 1:20000; Cat # sc8393; Santa Cruz), and rabbit anti-Cre recombinase (1:2000; Cat # PRB-106C; Covance).

For immunostaining, retinal whole-mounts or sections were blocked for 1 h in a solution containing 5% Chemiblocker (membrane-blocking agent, Chemicon), 0.5% Triton X-100 and 0.05% sodium azide (Sigma). The primary antibodies were diluted in the same solution and applied overnight, followed by incubation for 1h in the appropriate secondary antibody, which was conjugated to Alexa 555 (1:600; red fluorescence, Molecular Probes) or Alexa 488 (1:600; green fluorescence, Molecular Probes). All steps were carried out at room temperature (RT).

All images were generated using a Zeiss Axioplan 2 microscope with an Apotome oscillating grating to reduce out-of-focus stray light. Individual cells were selected, and Z-stack images

were captured using a Zeiss Apotome microscope. Image projections were made by collapsing individual z-stacks of optical sections into a single plane, unless otherwise indicated. The brightness and contrast were adjusted using Adobe Photoshop CS4.

Results

Cre-induced recombination or the expression of Cre recombinase was examined by crossing the Cre lines to a ROSA26 reporter mouse strain, which expresses EYFP upon Cre-mediated excision of a stop sequence, or by evaluating the expression of GFP in the lines with an integrated Cre-GFP transgene. Among the 15 Cre lines examined in this study, significant expression of EYFP or GFP in the retina was found in the majority of them (12 out of 15).

Cre-induced recombination in amacrine and ganglion cells

Grik4-cre—When viewed in retinal whole-mounts, EYFP-labeled cells in this line were found to tile throughout the entire retina (Figure 1A and B). The cell somas were located in the ganglion cell layer (GCL), and all of them were ganglion cells because each of them displayed an axon, as indicated by an arrow in Figure 1C (Also see left panel in Figure 1D). In retinal vertical sections, the dendrites of these cells were found to bistratify in the inner plexiform layer (IPL) (Left panel in Figure 1D). Furthermore, as revealed by co-staining with an antibody against ChAT, the dendrites of these cells showed co-stratification with the processes of starburst amacrine cells (Figure 1D). These results indicate that the Cre-induced recombination in this line is targeted to a class of bistratified ganglion cells.

ChAT-cre/Jax—In retinal whole-mount preparations, EYFP-labeled cells in this line were found to distribute throughout the entire retina. The cell somas were located in both the inner nuclear layer (INL) and GCL (Left panels of Figure 2A and 2B). These EYFP-positive cells were found to be starburst amacrine cells, the only cholinergic cells in the retina. This was demonstrated by double-labeling in retinal whole-mount preparations with an antibody against ChAT (Figure 2A and B). In both the INL and the GCL, all EYFP-expressing cells were immunoreactive for ChAT and vice versa. In retinal vertical sections, both displaced and regular starburst amacrine cell somas along with their dendrites were double-labeled for EYFP and ChAT (Figure 2C). Thus, these results indicate that Cre recombination is targeted exclusively to starburst amacrine cells in the retina in this line. In addition, all starburst amacrine cells appeared to express Cre recombinase.

ChAT-cre/Gsat—The expression of EYFP in this line was observed in cell somas located in the GCL and INL (Figure 3). Many of the cells located in the GCL were ganglion cells based on the presence of axons (Figure 3A). Those located in the INL are likely to be amacrine cells (Figure 3B). To determine if Cre recombination was present in cholinergic amacrine cells, the expression of EYFP was compared to ChAT immunoreactivity (Figure 3A and B, middle panels). The EYFP-positive cells were not found to be co-labeled by antibody to ChAT (Figure 3A and B, right panels), indicating that Cre recombinase is not present in cholinergic amacrine cells in this line. In vertical sections, the EYFP-labeled processes were found to be diffusely distributed throughout the IPL (Figure 3C, left panel). Again, no co-localization of ChAT and EYFP was found (Figure 3C).

Expression of Cre recombinase in retinal bipolar cells

Pcp2-cre/GFP and Chx10-cre/EGFP—Since both of these lines carry a GFP transgene in the Cre-transgenic construct, the expression of the Cre recombinase was examined based on GFP expression and antibody staining for Cre (see Methods).

When viewed in retinal whole-mounts, GFP-positive cells in the Pcp2-cre/GFP line were observed throughout the retina (Figure 4A). The cell somas were located in the INL (Figure 4A, left panel), and their axon terminals were found in the inner part of the IPL, close to the GCL (Figure 4A, right panel). In addition, weakly GFP-labeled cell somas were observed in the GCL (Figure 4A, right panel, pointed by an arrow). The processes of these cells could not be visualized, however, due to weak fluorescence. In vertical sections, the morphology of the brightly labeled cells resembled that of rod bipolar cells based on large somas located in the outer part of the INL and a few lobular axon terminals in the inner IPL (Figure 4C, left panel). This was further confirmed by double-labeling for protein kinase C (PKC, Figure 4C), as all GFP-positive cells were immunoreactive for PKC. Approximately $59 \pm 9\%$ of PKC-positive cells exhibited GFP expression ($n = 5$ retinal sections).

We examined another Cre line driven by the Pcp2 promoter, Tg(Pcp2-cre)2Mpin/J (Barski et al., 2000). It displayed very few GFP-labeled cells over the entire retina, however, which were probably bipolar cells (data not shown).

We also examined the expression of Cre recombinase in a Chx10-cre/GFP line, which was previously characterized by Cre/loxP recombination mainly in retinal bipolar cells (Rowan and Cepko, 2004). Like the Pcp2-cre/GFP line, in retinal whole-mounts the GFP-positive cells in the Chx10-cre/GFP line were observed throughout the retina (Figure 4B). The brightly GFP-labeled cell somas were located in the INL (Figure 4B, left panel), and their axon terminals were found in the inner part of the IPL, close to the GCL (Figure 4B, right panel). The density of GFP-expressing cells in this line was significantly higher than in the Pcp2-cre/GFP line. In vertical sections, the brightly GFP-labeled cells were rod bipolar cells, given that they co-localized with PKC (Figure 4D). On average, $89 \pm 13\%$ of PKC-positive cells showed GFP fluorescence ($n = 3$ sections). In addition, many weakly GFP-labeled cells were also observed. These cells are likely to be cone bipolar cells and Müller glial cells (see Rowan and Cepko, 2004). All of the GFP positive cells co-labeled with antibody to Cre (Figure 4E).

Expression of Cre recombinase in Müller glial cells

Foxg1-cre, Nes-cre, and Eno2-cre—In these three mouse lines, Cre-induced recombination was observed predominantly in Müller glial cells identified based on their morphological properties (Figure 5). Müller cells span the entire thickness of the retina. In the proximal retina, all three lines exhibited EYFP-fluorescence in the Müller cell endfeet at the inner limiting membrane (ILM) and processes surrounding the cells in the GCL (Figure 5A, 5F and 5K). In the middle of the retina, the somas of Müller cells were visualized in the INL (Figure 5B, 5G and 5L). The distal processes of these cells crossed the outer nuclear layer (ONL) and formed the outer limiting membrane (data not shown). To confirm the identity of these cells, retinal vertical sections were stained for glutamine synthetase (GS), a known marker of Müller cells (Figure 5, right three panels). In all three lines, cells with bright EYFP-fluorescence were positive for GS. In particular for the Foxg1 line, all EYFP-labeled cells exhibited strong fluorescence and showed immunoreactivity for GS. For the Nes-cre and NSE-cre lines, some cells with very weak EYFP-fluorescence were also observed in addition to the brightly EYFP-labeled Müller cells (Figure 5F and M, arrows). These weakly EYFP-labeled cells were located in the GCL and INL but not in the ONL. Based on the location of their somas and absence of GS immunoreactivity, these cells are likely to be ganglion, amacrine, and bipolar cells. Due to very low EYFP-fluorescence, we did not attempt to further identify these cells.

Cre-induced recombination in multiple retinal cell types

In the following four lines, EYFP labeling was observed in multiple retinal cell types, including Müller cells.

Thy-1-cre—The highest level of EYFP expression was observed in Müller cells, with sparse patches throughout the retina. In the GCL (Figure 6A), the brightest spots represented the endfeet of Müller cells. The weakly labeled cell somas were presumably ganglion cells. EYFP expression in the cell somas and processes of Müller cells was also observed in the INL, as shown in Figure 6B. In vertical sections (Figure 6C and D), bipolar, amacrine, and ganglion cells were observed. Meanwhile, EYFP-positive Müller cells were observed in small groups, unevenly distributed throughout the retina (Figure 6C).

Wnt1-cre—Sparse patches of Müller cells were strongly labeled in this line. In retinal whole-mount preparations (Figure 6E), the endfeet of Müller cells were observed in the GCL, in addition to some presumably ganglion cells and blood vessels. In the INL, strongly labeled somas of Müller cells along with some weakly labeled cells could be seen (Figure 6F). In vertical sections, these weakly labeled cells appeared to be bipolar cells and amacrine cells (Figure 6G).

Mnx1-cre—Sparse patches of Müller cells throughout the retina were also strongly labeled with EYFP. The endfeet could be seen in the proximal retina (Fig. 6H), and strongly-labeled somas in the INL (Figure 6I). In vertical sections, these weakly labeled cells in the INL could be clearly identified as bipolar cells and amacrine cells (Figure 6J).

CamK2a-cre—In retinal whole-mount preparations, EYFP-labeled cells were sparsely distributed throughout the retina (data not shown). In vertical sections, these cells were found to be bipolar cells, amacrine cells, and ganglion cells (Figure 6K). No EYFP expression was observed in Müller cells.

Only a few cells (<5), probably astrocytes, were observed in each retina of the *Emx1-cre* line (data not shown). No Cre-mediated recombination was detected in the retina of the *Nr5a1-cre* line.

Discussion

In this study, we examined the expression patterns of Cre-induced recombination or Cre recombinase in the retinas of 15 Cre mouse lines. Significant Cre-induced recombination or expression of Cre recombinase was observed in the majority of these lines. A summary of the results for the 15 Cre mouse lines is shown in Table 1.

The *Grik4-cre* line was produced using a BAC clone containing the promoter sequence for one of kainite receptor subunits, KA-1. In the brain, the Cre-induced recombination was reported in the neurons of area CA3 of the hippocampus where the high expression of this receptor has been expected (Nakazawa et al., 2002). In the retina of this line, we found that Cre-induced recombination occurred in a unique class of retinal ganglion cells, bistratified ganglion cells. Since these cells were found to tile throughout the retina, they likely represent a single type of retinal ganglion cells. Two types of bistratified ganglion cells have been reported in the mouse retina (Sun et al., 2002). One of them has been proposed to be ON-OFF directional selective ganglion cells (DSGCs). The dendrites of the ON-OFF DSGCs have been shown to co-stratify with the processes of starburst amacrine cells (Famiglietti, 1992; Vaney and Pow, 2000). Our co-labeling results show that the dendrites of the Cre-expressing cells in the *Grik4-cre* line co-stratify with the processes of starburst amacrine cells. Thus, these Cre-expressing cells are very likely to be ON-OFF DSGCs. Four subtypes of ON-OFF DSGCs sensitive to one of four motion directions have been proposed (Vaney, 1994; Amthor and Oyster, 1995; Demb, 2007). More recently, a subtype of ON-OFF DSGCs labeled by GFP in a transgenic line was reported (Huberman et al., 2009). These DSGCs were found to direct posterior motion and showed

unique central projections. Thus, it will be very interesting to examine the physiological properties and central projections of these Cre-expressing ganglion cells in further studies.

It remains unknown whether the targeting of Cre recombinase to this class of ganglion cells is due to the expression of the KA-1 gene. Although expression of the KA-1 gene has been reported in the retina (Duvoisin et al., 1995; Jakobs et al., 2007; Kwon et al., 2007; Hanna and Calkins, 2007; but see Brandstätter et al., 1994), such a unique expression pattern has not been reported. Therefore, it would not be surprising that the expression of Cre recombinase in these ganglion cells were due to a position effect, despite the fact that this mouse line was generated using a BAC clone. Nevertheless, this mouse line with such abnormal targeting turned out to be a valuable animal model.

The Cre-induced recombination in the ChAT-cre/Jax mouse line was found to target the cholinergic or starburst amacrine cells in the retina. Based on immunostaining with the ChAT antibody, all of the starburst amacrine cells, both regular and displaced, were found to be positive for EYFP and vice versa. Thus, the Cre recombinase in this line targets exclusively to all starburst amacrine cells. The targeting of ChAT-cre to starburst amacrine cells was expected because starburst amacrine cells are the only cholinergic cells in the retina (Masland & Mills, 1979), and this line was generated by knock-in method (see the description of the Jackson Laboratory). It is worth mentioning, however, that although this Cre line generated by a knock-in method only carries one copy of the Cre-gene in the ChAT locus, our results indicate the high efficiency of Cre-induced recombination in the retina.

In contrast, the Cre recombination of the ChAT-cre/Gsat line, produced using a BAC clone, was not found to target cholinergic or starburst amacrine cells. Instead, Cre recombination was observed in other amacrine cells as well as in ganglion cells. BAC genomic clones have been used to produce transgenic mice with selective targeting (Gong et al., 2003). Interestingly, the expression of Cre-induced recombination of this line has been reported to match the pattern of cholinergic neurons in the brain (Gong et al., 2007). Clearly, this was not the case in the retina. A recent study of a ChAT-EGFP transgenic line produced using a BAC clone also found that EGFP was not targeted to cholinergic amacrine cells in the retina (Haverkamp et al., 2009). On the other hand, since Cre-induced recombination in the ChAT-cre/Gsat line was observed in some populations of amacrine and ganglion cells, this Cre line could be useful for genetic manipulations or targeting in third-order retinal neurons.

Starburst amacrine cells have been known to play a central role in retinal directional selectivity through asymmetric inhibition of DSGCs (Fried et al., 2002; Lee and Zhou, 2006; Demb, 2007). A model based on differential expression of two different chloride transporters has been proposed to generate this asymmetric inhibition (Gavrikov *et al.* 2003, 2006). The ChAT-cre/Jax lines could be used to dissect the molecular mechanisms of starburst amacrine cells in retinal direction selectivity.

In this study, we also examined two cre-lines, Tg(Pcp2-cre)1Amc/J (or Pcp2-cre/GFP) and Tg(Pcp2-cre)2Mpin/J, in which the Cre recombinase was driven by the Pcp2 promoter. The transgenic lines using this promoter have been known to target Purkinje cells in the cerebellum and rod bipolar cells in the retina (Oberdick et al., 1990). Consistent with this, in the Tg(Pcp2-cre)1Amc/J mouse line, which was generated by a transgenic construct of Pcp2, the majority of Cre/GFP-positive cells were rod bipolar cells, although some cells located in the GCL were also found to express Cre/GFP. Based on immunolabeling for PKC, about 60% of rod bipolar cells were targeted by Cre recombinase. On the other hand, in Tg(Pcp2-cre)2Mpin/J, which was generated using a knock-in method, only a few cells expressing Cre recombinase, possibly bipolar cells, were found in the retina. It is interesting to note that the expression of Cre in Purkinje cells in the cerebellum appeared to be much stronger in the Tg(Pcp2-cre)2Mpin/J line

than in the Tg(Pcp2-cre)1Amc/J line (see description by The Jackson Laboratory). Previously, a number of Cre lines using Pcp2 promoter were also reported. A Cre line generated using a BAC clone was reported to target exclusively rod bipolar cells in the retina, but the expression of Cre was developmentally dependent (Zhang et al., 2005). Another line using a short Pcp2 promoter only yielded expression of Cre recombinase in a few bipolar cells (Barski et al., 2000). In addition, several Cre lines produced using a knock-in method showed varied expression patterns in photoreceptor cells and bipolar cells in the retina (Saito et al., 2005). In one of these lines, Cre-induced recombination targeted 43% of rod bipolar cells. Thus, the expression pattern of Pcp2 promoter-driven Cre recombinase appears to be varied not only among transgenic lines but also among knock-in lines.

For comparison, we also examined the expression of Cre recombinase in bipolar cells in one of the Chx10-cre/GFP lines (line 2; Rowan and Cepko, 2004). Consistent with the earlier report, the vast majority of the Cre/GFP-positive cells were bipolar cells. In particular, we showed that about 90% of rod bipolar cells were targeted by Cre/GFP. In addition, Cre expression, albeit much weaker, was found in a significant number of cone bipolar cells. Both ON and OFF cone bipolar cells were reported to be targeted in another Chx10-cre/GFP line (line 1; Rowan and Cepko, 2004).

Thus, the Tg(Pcp2-cre)1Amc/J line could be used to target rod bipolar cells, whereas the Chx10-cre/GFP line to target both rod and cone bipolar cells.

In three Cre lines, Foxg1-cre, Nes-cre, and Eno2-cre, Cre-induced recombination was predominantly targeted to Müller glial cells in the retina. Among these three lines, Cre recombinase in the Foxg1-cre line appears to target exclusively Müller glial cells. Also in this line, the vast majority – if not all – of the Müller glial cells were found to be Cre-positive. On the other hand, for the Nes-cre and Eno2-cre lines, weak cre-induced recombination was found in retinal neurons. Müller glial cells have been known to play an important role in supporting normal retinal neuronal functions, and Müller glial cell dysfunction may underlie many retinal diseases and disorders (Bringmann et al., 2006). Thus, Müller glial cells could be an excellent target for retinal gene therapy. These Cre lines, especially the Foxg1-cre line, should be useful for Müller glial cell-specific gene targeting.

The expression of Cre recombinase in Thy-1-cre, Wnt1-cre, Mnx1-cre, and Camk2a lines was also found in multiple retinal cell types, including Müller glial cells. The populations of Cre recombinase-positive cells and their densities were varied among these lines, which could be useful if gene manipulation in multiple retinal cell types is desired.

In summary, we identified several lines with Cre recombinase expression exclusively or predominantly targeted to a single type or class of retinal cells, including bistratified retinal ganglion cells, starburst amacrine cells, rod bipolar cells, and Müller glial cells. In addition, in several other lines, the Cre-induced recombination or Cre recombinase was found in multiple retinal cell types. These Cre lines provide a valuable resource for the use of Cre-LoxP technology in retinal research.

The increasing availability of Cre-transgenic lines and the database of the Cre-recombinase expression patterns in the retina will make it possible to have a wide variety of animal models with Cre recombinase expression in any specific cell type(s) in the retina. Thus, the Cre/LoxP system is expected to become an extremely powerful tool in retinal research as well. An increasing number of floxed conditional gene knockout mouse lines will be available (see KnockOut Mouse Project, <http://www.knockoutmouse.org>; European Conditional Mouse Mutagenesis Program, <http://www.eucomm.org>; North American Conditional Mouse Mutagenesis Project, <http://norcomm.phenogenomics.ca/index.htm>), which will allow the deletion of endogenous genes in specific cells in the retina. Combining the Cre/LoxP system

with tetracycline-inducible expression systems could further allow precise spatiotemporal regulation of gene expression (Belteki et al., 2005; Yu et al., 2005). Furthermore, manipulation of endogenous gene expression or targeting expression of an exogenous gene in specific retinal cells can be achieved by viral vectors carrying floxed transgenes (Schnütgen et al., 2003; Kuhlman and Huang 2008; Atasoy et al., 2008). Thus, the Cre-transgenic mouse lines can be used to dissect retinal structure and function, as well as to generate animal models for studying and treating human retinal diseases.

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Abbreviations

EYFP	enhanced yellow fluorescent protein
GFP	green fluorescent protein
BAC	bacterial artificial chromosome
ONL	outer nuclear layer
INL	inner nuclear layer
IPL	inner plexiform layer
GCL	ganglion cell layer
KA-1	kainate receptor subunit 1
ChAT	choline acetyl transferase
Pcp2	Purkinje cell protein 2
Thy-1	thymocyte differentiation antigen 1

References

- Akimoto M, Filippova E, Gage PJ, Zhu X, Craft CM, Swaroop A. Transgenic mice expressing Cre-recombinase specifically in M- or S-cone photoreceptors. *Invest Ophthalmol Vis Sci* 2004;45:42–47. [PubMed: 14691152]
- Amthor FR, Oyster CW. Spatial organization of retinal information about the direction of image motion. *Proc Natl Acad Sci U S A* 1995;92:4002–4005. [PubMed: 7732021]
- Arber S, Han B, Mendelsohn M, Smith M, Jessell TM, Sockanathan S. Requirement for the homeobox gene Hb9 in the consolidation of motor neuron identity. *Neuron* 1999;23:659–674. [PubMed: 10482234]
- Atasoy D, Aponte Y, Su HH, Sternson SM. A FLEX switch targets Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. *J Neurosci* 2008;28:7025–7030. [PubMed: 18614669]
- Barski JJ, Dethleffsen K, Meyer M. Cre recombinase expression in cerebellar purkinje cells. *Genesis* 2000;28:93–98. [PubMed: 11105049]
- Belteki G, Haigh J, Kabacs N, Haigh K, Sison K, Costantini F, Whitsett J, Quaggin SE, Nagy A. Conditional and inducible transgene expression in mice through the combinatorial use of Cre-mediated recombination and tetracycline induction. *Nucleic Acids Res* 2005;33:e51. [PubMed: 15784609]
- Branda CS, Dymecki SM. Talking about a revolution: The impact of site-specific recombinases on genetic analyses in mice. *Dev Cell* 2004;6:7–28. [PubMed: 14723844]

- Brandstätter JH, Hartveit E, Sassoe-Pognetto M, Wässle H. Expression of NMDA and high-affinity kainate receptor subunit mRNAs in the adult rat retina. *Eur J Neurosci* 1994;6:1100–1112. [PubMed: 7952290]
- Bringmann A, Pannicke T, Grosche J, Francke M, Wiedemann P, Skatchkov SN, Osborne NN, Reichenbach A. Müller cells in the healthy and diseased retina. *Prog Retin Eye Res* 2006;25:397–424. [PubMed: 16839797]
- Danielian PS, Muccino D, Rowitch DH, Michael SK, McMahon AP. Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. *Curr Biol* 1998;8:1323–1326. [PubMed: 9843687]
- Demb JB. Cellular mechanisms for direction selectivity in the retina. *Neuron* 2007;55:179–186. [PubMed: 17640521]
- Dewachter I, Reverse D, Caluwaerts N, Ris L, Kuiperi C, Van den Haute C, Spittaels K, Umans L, Serneels L, Thiry E, Moechars D, Mercken M, Godaux E, Van Leuven F. Neuronal deficiency of presenilin 1 inhibits amyloid plaque formation and corrects hippocampal long-term potentiation but not a cognitive defect of amyloid precursor protein [V717I] transgenic mice. *J Neurosci* 2002;22:3445–3453. [PubMed: 11978821]
- Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, Kenny CD, Christiansen LM, White RD, Edelstein EA, Coppari R, Balthasar N, Cowley MA, Chua S Jr, Elmquist JK, Lowell BB. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 2006;49:191–203. [PubMed: 16423694]
- Duvoisin RM, Zhang C, Hamassaki-Britto DF, Britto LRG. Changes in expression of glutamate receptor subunits following photoreceptor degeneration in the rd mouse retina. *Neurosci Letters* 1995;183:83–86.
- Famiglietti EV. Dendritic co-stratification of ON and ON-OFF directionally selective ganglion cells with starburst amacrine cells in rabbit retina. *Journal of Comparative Neurology* 1992;324:322–335. [PubMed: 1383291]
- Forss-Petter S, Danielson PE, Catsicas S, Battenberg E, Price J, Nerenberg M, Sutcliffe JG. Transgenic mice expressing b-galactosidase in mature neurons under neuron-specific enolase promoter control. *Neuron* 1999;5:187–197. [PubMed: 2116814]
- Fried SI, Munch TA, Werblin FS. Mechanisms and circuitry underlying directional selectivity in the retina. *Nature* 2002;420:411–414. [PubMed: 12459782]
- Frugier T, Tiziano FD, Cifuentes-Diaz C, Miniou P, Roblot N, Dierich A, Le Meur M, Melki J. Nuclear targeting defect of SMN lacking the C-terminus in a mouse model of spinal muscular atrophy. *Hum Mol Genet* 2000;9:849–858. [PubMed: 10749994]
- Gavériaux-Ruff C, Kieffer BL. Conditional gene targeting in the mouse nervous system: Insights into brain function and diseases. *Pharmacol Ther* 2007;113:619–634. [PubMed: 17289150]
- Gavrikov KE, Dmitriev AV, Keyser KT, Mangel SC. Cation chloride cotransporters mediate neural computation in the retina. *Proc Natl Acad Sci U S A* 2003;100:16047–16052. [PubMed: 14665697]
- Gavrikov KE, Nilson JE, Dmitriev AV, Zucker CL, Mangel SC. Dendritic compartmentalization of chloride cotransporters underlies directional responses of starburst amacrine cells in retina. *Proc Natl Acad Sci U S A* 2006;103:18793–18798. [PubMed: 17124178]
- Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 2003;425:917–925. [PubMed: 14586460]
- Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* 2007;27:9817–9823. [PubMed: 17855595]
- Gorski JA, Talley T, Qiu M, Puellas L, Rubenstein JL, Jones KR. Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J Neurosci* 2002;22:6309–6314. [PubMed: 12151506]
- Hanna MC, Calkins JC. Expression of genes encoding glutamate receptors and transporters in rod and cone bipolar cells of the primate retina determined by single-cell polymerase chain reaction. *Molecular Vision* 2007;13:2194–2208. [PubMed: 18087239]

- Haverkamp S, Inta D, Monyer H, Wässle H. Expression analysis of green fluorescent protein in retinal neurons of four transgenic mouse lines. *Neurosci* 2009;160:126–139.
- Hebert JM, McConnell SK. Targeting of cre to the Foxg1 (BF-1) locus mediates loxP recombination in the telencephalon and other developing head structures. *Dev Biol* 2000;222:296–306. [PubMed: 10837119]
- Huberman AD, Wei W, Elstrott J, Stafford BK, Feller MB, Barres BA. Genetic identification of an On-Off direction-selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. *Neuron* 2009;62:327–334. [PubMed: 19447089]
- Jakobs TC, Ben Y, Masland RH. Expression of mRNA for glutamate receptor subunits distinguishes the major classes of retinal neurons, but is less specific for individual cell types. *Mol Vis* 2007;13:933–948. [PubMed: 17653033]
- Jimeno D, Feiner L, Lillo C, Teofilo K, Goldstein LS, Pierce EA, Williams DS. Analysis of kinesin-2 function in photoreceptor cells using synchronous Cre-loxP knockout of Kif3a with RHO-Cre. *Invest Ophthalmol Vis Sci* 2006;47:5039–5046. [PubMed: 17065525]
- Kuhlman SJ, Huang ZJ. High-resolution labeling and functional manipulation of specific neuron types in mouse brain by Cre-activated viral gene expression. *PLoS One* 2008;3:e2005. [PubMed: 18414675]
- Kwon O-J, Kim M-S, Kim T-J, Jeon C-J. Identification of synaptic pattern of kainate glutamate receptor subtypes on direction-selective retinal ganglion cells. *Neurosci Res* 2007;58:255–264. [PubMed: 17466402]
- Le YZ, Ash JD, Al-Ubaidi MR, Chen Y, Ma JX, Anderson RE. Targeted expression of Cre recombinase to cone photoreceptors in transgenic mice. *Mol Vis* 2004;10:1011–1018. [PubMed: 15635292]
- Le YZ, Zheng L, Zheng W, Ash JD, Agbaga MP, Zhu M, Anderson RE. Mouse opsin promoter-directed Cre recombinase expression in transgenic mice. *Mol Vis* 2006;12:389–398. [PubMed: 16636658]
- Lee S, Zhou ZJ. The synaptic mechanism of direction selectivity in distal processes of starburst amacrine cells. *Neuron* 2006;51:787–799. [PubMed: 16982423]
- Lewis PM, Gritli-Linde A, Smeyne R, Kottmann A, McMahon AP. Sonic hedgehog signaling is required for expansion of granule neuron precursors and patterning of the mouse cerebellum. *Dev Biol* 2004;270:393–410. [PubMed: 15183722]
- Li S, Chen D, Sauve Y, McCandless J, Chen YJ, Chen CK. Rhodopsin-iCre transgenic mouse line for Cre-mediated rod-specific gene targeting. *Genesis* 2005;41:73–80. [PubMed: 15682388]
- Masland RH, Mills JW. Autoradiographic identification of acetylcholine in the rabbit retina. *J Cell Biol* 1979;83:159–178. [PubMed: 92476]
- Masland RH. The fundamental plan of the retina. *Nat Neurosci* 2001;4:877–886. [PubMed: 11528418]
- Nagy A. Cre recombinase: the universal reagent for genome tailoring. *Genesis* 2000;26:99–109. [PubMed: 10686599]
- Nakazawa K, Quirk MC, Chitwood RA, Watanabe M, Yeckel MF, Sun LD, Kato A, Carr CA, Johnston D, Wilson MA, Tonegawa S. Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science* 2002;297:211–218. [PubMed: 12040087]
- Oberdick J, Smeyne RJ, Mann JR, Zackson S, Morgan JI. A promoter that drives transgene expression in cerebellar Purkinje and retinal bipolar neurons. *Science* 1990;248:223–226. [PubMed: 2109351]
- Rowan S, Cepko CL. Genetic analysis of the homeodomain transcription factor Chx10 in the retina using a novel multifunctional BAC transgenic mouse reporter. *Dev Biol* 2004;271:388–402. [PubMed: 15223342]
- Saito H, Tsumura H, Otake S, Nishida A, Furukawa T, Suzuki N. L7/Pcp-2-specific expression of Cre recombinase using knock-in approach. *Biochem Biophys Res Commun* 2005;331:1216–1221. [PubMed: 15883005]
- Schnütgen F, Doerflinger N, Calléja C, Wendling O, Chambon P, Ghyselinck NB. A directional strategy for monitoring Cre-mediated recombination at the cellular level in the mouse. *Nat Biotechnol* 2003;21:562–565. [PubMed: 12665802]
- Srinivas S, Watanabe T, Lin CS, Williams CM, Tanabe Y, Jessell TM, Costantini F. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* 2001;1:4. [PubMed: 11299042]

- Sun W, Li N, He S. Large-scale morphological survey of rat retinal ganglion cells. *Vis Neurosci* 2002;19:483–493. [PubMed: 12511081]
- Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, Bock R, Klein R, Schutz G. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat Genet* 1999;23:99–103. [PubMed: 10471508]
- Tsien JZ, Chen DF, Gerber D, Tom C, Mercer EH, Anderson DJ, Mayford M, Kandel ER, Tonegawa S. Subregion- and cell type-restricted gene knockout in mouse brain. *Cell* 1996;87:1317–1326. [PubMed: 8980237]
- Vaney DI. Territorial organization of direction-selective ganglion cells in rabbit retina. *J Neurosci* 1994;14:6301–6316. [PubMed: 7965037]
- Vaney DI, Pow DV. The dendritic architecture of the cholinergic plexus in the rabbit retina: selective labeling by glycine accumulation in the presence of sarcosine. *J Comp Neurol* 2000;421:1–13. [PubMed: 10813769]
- Yu HM, Liu B, Chiu SY, Costantini F, Hsu W. Development of a unique system for spatiotemporal and lineage-specific gene expression in mice. *Proc Natl Acad Sci USA* 2005;102:8615–8620. [PubMed: 15941831]
- Wässle H. Parallel processing in the mammalian retina. *Nat Rev Neurosci* 2004;5:747–757. [PubMed: 15378035]
- Zimmerman L, Lendahl U, Cunningham M, McKay R, Prr B, Gavin B, Mann J, Vassileva G, McMahon A. Independent regulation of elements in the nestin gene direct transgene expression to neural stem cells or muscle precursors. *Neuron* 1994;12:11–24. [PubMed: 8292356]
- Zhang XM, Chen BY, Ng AH, Tanner JA, Tay D, So KF, Rachel RA, Copeland NG, Jenkins NA, Huang JD. Transgenic mice expressing Cre-recombinase specifically in retinal rod bipolar neurons. *Invest Ophthalmol Vis Sci* 2005;46:3515–3520. [PubMed: 16186328]

Figure 1.

Grik4-cre mouse line expresses Cre recombinase in a population of ganglion cells. (A–C) The expression of EYFP viewed in the GCL/IPL in a retinal whole-mount at low (A) and high (B) magnification. (C) EYFP-labeled axons viewed in the GCL/NFL. (D) Vertical section shows the stratification of the dendrites of an EYFP-positive ganglion cell (left panel) in comparison with ChAT-labeled bands (middle panel). The merged image is shown in the right panel in (D). The arrows in (C) and in the left panel in (D) indicate the axon of the ganglion cell. Scale bars: 50 μ m in A, 25 μ m in B–D. INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; NFL: nerve fiber layer.

Figure 2.

Expression of Cre recombinase in starburst amacrine cells in the ChAT-cre/Jax mouse line. The retina was photographed in a whole-mount in the GCL (**A**), the INL (**B**, the same region as in **A**), and a vertical section (**C**). The retina was double-labeled for EYFP (left panels) and ChAT (middle panels). Superimpositions are shown in the right panels. Scale bars: 100 μ m in A and B, 25 μ m in C. OPL, outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer.

Figure 3.

Expression of Cre recombinase in the ChAT-cre/Gsat mouse line. (**A–B**) A whole-mount retina was double-labeled for EYFP (left panels) and ChAT (middle panels). Merged images (right panels) revealed no co-localization in either the GCL (**A**) or the INL (**B**). (**C**) In a vertical section, EYFP-positive cells (left panel) did not exhibit ChAT immunofluorescence (middle panel). The merged image demonstrates the absence of co-localization (right panel). Scale bars: 100 μ m in A and B, 25 μ m in C. OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer.

Figure 4.

The expression of Cre recombinase in bipolar cells. (**A–B**) The cell somas of bipolar cells were viewed in retinal whole-mounts of PCP2-cre/GFP (**A**) and Chx10-cre/GFP (**B**) mice in the INL (left panels), and their axon terminals, in the IPL (right panels). For the PCP2-cre/GFP line, some weakly labeled cells located in the GCL were encountered (arrow). (**C–D**) Retinal vertical sections of PCP2-cre/GFP (**C**) and Chx10-cre/GFP (**D**) mice were double-labeled for GFP (left panels) and PKC (middle panels), with merged images shown in the right panels. (**E**) A retinal section from a Chx10-cre/GFP mouse was stained for GFP (left panel) and Cre (middle panel), and co-localization is shown in a merged image (right panel). Scale bars: 100 μ m in A and B, 25 μ m in C–E. OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; bv: blood vessel.

Figure 5.

Mouse lines with predominant expression of Cre recombinase in Müller glial cells. Whole-mount retinas of Foxg1-cre (upper panels), NSE-cre (middle panels) and Eno2-cre (lower panels) lines were analyzed in the same regions in the GCL (**A**, **F** and **K**) and the INL (**B**, **G** and **L**). Retinal vertical sections were immunostained for EYFP (**C**, **H** and **M**) and GS (**D**, **I** and **N**), and superimposed images were created (**E**, **J** and **O**). In addition to Müller cells, some weakly labeled neurons were encountered in the NSE-cre and Eno2-cre lines (arrows). Scale bars: 50 μ m for whole-mounts (**A** and **B**, **F** and **G**, **K** and **L**), 25 μ m for retinal sections (**C**–**E**, **H**–**J**, **M**–**O**). INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer.

Figure 6.

Mouse lines with expression of Cre recombinase in multiple retinal cell types. (A–D) In the Thy1-cre mouse line, strong EYFP expression was detected in sparse patches of endfeet (A) and somas (B) of Müller cells, as well as some weakly labeled neurons. (C–D) Vertical sections showed the region with patches of EYFP-labeled Müller cells (C) and the region where only EYFP-labeled neurons were observed (D). (E–G) In the Wnt1-cre mouse line, high EYFP expression was detected in Müller cells in the GCL (E) and the INL (F). Blood vessels also appeared to be labeled (E). In the GCL and INL, some weakly labeled somas were encountered. A characteristic view of a vertical section in which Müller cells and weakly labeled bipolar cells and amacrine cells can be seen (G). (H–J) In the Mnx1-cre mouse line, strong EYFP fluorescence was observed in Müller cells in the GCL (H) and in the INL (I) in a retinal whole-mount. The weak EYFP expression was detected in bipolar cells and amacrine cells in vertical sections (J). (K) In the CamK2a-cre mouse line, weak EYFP fluorescence was detected sparsely in different types of retinal neurons but not in Müller cells. Scale bars: 50 μ m in A, B, E, F, H and I, 25 μ m in C, D, G, J and K. OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer.

Table 1

Mouse lines expressing Cre-recombinase in the retina.

Mouse line	Retinal cells expressing Cre-recombinase	
	Strong expression	Weak expression
1. Grik4-cre	bistratified ganglion cells	
2. ChAT-cre/Jax	all cholinergic amacrine cells	
3. ChAT-cre/Gsat	ganglion and amacrine cells	
4. Pcp2-cre/GFP	rod bipolar cells	ganglion cells
5. Pcp2-cre/2Mpin/J	probably bipolar cells (very few)	
6. Chx10-cre/EGFP	rod bipolar cells	cone bipolar and Müller cells
7. Foxg1-cre	Müller cells (evenly)	
8. Nes-cre	Müller cells (evenly)	inner retinal neurons *
9. Eno2-cre	Müller cells (evenly)	inner retinal neurons *
10. Thy-1-cre	Müller cells (sparse, patchy)	inner retinal neurons *
11. Wnt1-cre	Müller cells (sparse, patchy)	inner retinal neurons *
12. Mnx1-cre	Müller cells (sparse, patchy)	inner retinal neurons *
13. CamK2a-cre	inner retinal neurons * (sparse)	
14. Emx1-cre	probably astrocytes (very few)	
15. Nr5a1-cre	no expression	

* inner retina neurons include ganglion, amacrine, and bipolar cells.