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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 become 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 central nervous system; 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.

Key words: Cre-transgenic mice, green fluorescent protein, retina, bipolar cell, amacrine cell, ganglion cell.

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.

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 become a powerful tool, allowing gene deletion, over-expression, and ectopic expression *in vivo* in a cell- and tissue-specific fashion (Nagy, 2000; Branda and Dymecki, 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 choline acetyl transferase (ChAT) gene (see the description of the Jackson Laboratory); (3) Tg(ChAT-cre)24Gsat (ChAT-cre/Gsat),

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Abbreviations: BAC, bacterial artificial chromosome; Camk2a, calcium/calmodulin-dependent protein kinase II alpha; ChAT, choline acetyl transferase; Chx10, C. elegans ceh-10 homeo domain; CNS, central nervous system; DSGC, directional selective ganglion cell; Emx1, Drosophila empty homolog 1; Eno2, enolase 2; EYFP, enhanced yellow fluorescent protein; Foxg1, Forkhead box G1; GCL, ganglion cell layer; GFP, green fluorescent protein; Grik4, gene encoding kainite receptor 1; GS, glutamine synthetase; INL, inner nuclear layer; IPL, inner plexiform layer; KA-1, kainate receptor subunit 1; MNX1, motor neuron and pancreas homeobox 1; Nes, nestin; NFL, nerve fiber layer; Nr5a1, steroidogenic factor-1; OPL, outer plexiform layer; PB, phosphate buffer; Pcp2, Purkinje cell protein 2; PKC, protein kinase C; Thy-1, thymocyte differentiation antigen 1; Wnt1, wingless type mouse mammary tumor virus integration site gene family member one (Wnt-1).

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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(Purkinje cell protein 2 [Pcp2]-cre)1Amc/J (Pcp2-cre/green fluorescent protein [GFP]), generated by driving Cre recombinase and GFP or a GFP derivative under the control of a modified mouse 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 four 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 enhanced green fluorescent protein (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 and McConnell, 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 2 (Eno2) gene promoter (Frugier et al., 2000; Forss-Petter, 1999); (10) FVB/N-Tg(thymocyte differentiation antigen 1[Thy]-1-cre)1Vln/J (Thy-1-cre), generated by driving the Cre recombinase gene under the control of the mouse 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 one (Wnt-1) 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$^{tm1(cre)Krj}$/J (Em×1-cre), generated by inserting an IRES-Cre gene into the exon encoding the 3′ untranslated region of the mouse homeobox gene Em×1 (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- enhanced yellow fluorescent protein (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 was evaluated in the Cre-positive offspring at 1 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 CO_2 and decapitated. The retinas were fixed in the eyecups with 4% paraformaldehyde in 0.1 M phosphate buffer (PB) for 20 min. 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. 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- ChAT (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-106 C; 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 1 h 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 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 (Fig. 1A, 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 Fig. 1C (Also see left panel in Fig. 1D). In retinal vertical sections, the dendrites of these cells were found to bistratify in the inner plexiform layer (IPL) (Left panel in Fig. 1D). Furthermore, as revealed by co-staining with an antibody against ChAT, the dendrites of these cells showed co-stratification with the processes of