FEBS Letters 580 (2006) 1479–1484

Conserved *cis*-elements in the *Xenopus* red opsin promoter necessary for cone-specific expression

S. Babu[1], V. McIlvain[1], S.L. Whitaker[2], B.E. Knox*

Department of Biochemistry & Molecular Biology and Ophthalmology, 750 E. Adams Street, SUNY Upstate Medical University, Syracuse, NY 13210, United States

Received 27 December 2005; revised 24 January 2006; accepted 25 January 2006

Available online 2 February 2006

Edited by Gianni Cesareni

Abstract The long-wavelength sensitive (red) opsin genes encode proteins which play a central role in daytime and color vision in vertebrates. We used transgenic *Xenopus* to identify 5′ *cis*-elements in the red cone opsin promoter necessary for cone-specific expression. We found a highly conserved extended region (−725 to −173) that was required for restricting GFP transgene expression to cones. We further identified a short element (5′-CCAATTAAG-AGAT-3′) highly conserved amongst tetrapods, including humans, necessary to restrict expression to cones in the retina. These results identify novel conserved elements that regulate spatial expression of tetrapod red cone opsin genes.

Keywords: Retina; Transcription; Gene expression; Photoreceptor; Promoter; Transgenic

1. Introduction

Visual pigments, composed of opsin and bound retinal chromophore, absorb photons and are classified by sequence similarity and spectral absorption properties [1–5]. In vertebrates there are five opsin families: rhodopsin (RH1), middle/long-wavelength sensitive (M/LWS), rhodopsin-like middle wavelength sensitive (RH2) and two short wavelength sensitive (SWS) opsins, SWS1 and SWS2. M/LWS opsins are central to vertebrate color vision. They are the dominant visual pigments in the human central retina and are essential for high acuity and daytime vision [6]. The mechanisms that regulate cone opsin gene expression are not as well characterized as for the rod opsin pigments. In both photoreceptor types, gene expression is largely controlled by transcriptional mechanisms which determine cell-specificity [7], developmental timing [8–10] and circadian rhythms [11,12]. For the vertebrate rhodopsin gene, extensive effort has uncovered novel transcription factors, *cis*-elements and a relatively compact promoter structure [13–20]. A striking feature is the conservation of regulatory mechanisms amongst vertebrates, both at the structural and functional levels [14]. Most significantly, the transcription factors neural retina leucine zipper (Nrl), cone–rod homeobox (Crx) and photoreceptor specific orphan nuclear receptor (NR2E3) are essential regulators of rhodopsin transcription levels [20–23]. It seems likely that vertebrate cones may also share similar regulatory mechanisms [24].

Pioneering studies are beginning to address questions regarding transcriptional regulation of M/LWS genes [25–27]. In humans, mutually exclusive expression of red and green opsins determines cell specificity and is regulated by an upstream locus control region (LCR). The current model suggests that proximal promoters compete stochastically for the LCR [25,26]. However, the structure of the proximal promoter is not well characterized with only a few potential *cis*-elements identified in a heterologous system [28]. Using transgenic *Xenopus* approaches, we have previously analyzed a number of retinal-specific promoters [16,17,24,29–32]. We describe here the analysis of the *Xenopus* LWS promoter.

2. Materials and methods

2.1. Nucleic acids

A *Xenopus laevis* BAC clone (94N23, Research Genetics/Invitrogen, Carlsbad, California) was chosen to isolate the upstream region of the LWS opsin. Nucleotides 19–174 of exon 1 matched the published cDNA sequence [9]. The upstream sequence was found to be 99% similar to a *Xenopus* LWS cone opsin 5′ region previously cloned using RAGE [33]. The translational initiation site was mutated to a *Kpn*I site (QuikChange, Stratagene) and the resulting upstream region (−1792/+127) was cloned upstream of EGFP in pEGFP(−) vector [17]. Four deletion constructs and targeted disruptions were made by PCR. All constructs were sequenced prior to preparing transgenics as previously described [14].

2.2. Sequence analyses

The upstream sequences were obtained from http://www.ensembl.org/index.html (human red, accession #ENSG00000102076) and from JGI V3.0 (http://genome.jgi-psf.org/Xentr4/Xentr4.home.html). The Databases of Orthologous Promoters (DoOP) Database1.1 [34] was queried with the human red opsin ID. Sequence alignments, including the two *Xenopus*, four teleosts sequences (*Danio rerio* LWS1&2, *Tetradon nigroviridis* and *Fugu rubripes*) and the eight mammalian species in the DoOP database were performed using ClustalW algorithm (DNAStar, Lasergene). Potential *cis*-elements were identified by TESS/TRANSFAC 4.0 (www.cbil.upenn.edu/tess).

*Corresponding author. Fax: +1 315 464 8750.
E-mail address: knoxb@upstate.edu (B.E. Knox).

[1] These authors contributed equally.
[2] Present address: Department of Ophthalmology, 3333 Burnett Avenue, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229-3039, United States.

Abbreviations: GFP, green fluorescent protein; M/LWS, middle/long-wavelength sensitive; SWS, short wavelength sensitive; ROP, red opsin promoter elements

0014-5793/$32.00
doi:10.1016/j.febslet.2006.01.080

2.3. Transgenic Xenopus

Transgenic *Xenopus* was produced by restriction enzyme mediated integration [14,35]. Live transgenic animals were screened for green fluorescent protein (GFP) expression and fixed in 4% paraformaldehyde for 2 h prior to embedding for cryosectioning. Sections (12–14 μm thickness) were prepared and stained with either monoclonal anti-rhodopsin (4D2) [36] or anti-calbindin (Oncogene) antibodies.

3. Results

3.1. Cone-specific expression in transgenic Xenopus

We isolated exon 1 and 1.8 kb of 5′ upstream fragment of the *Xenopus* LWS opsin gene from a BAC library and determined the transcription start site (data not shown). This fragment (−1792/+127) was used to direct GFP reporter expression in transgenic *Xenopus* [14,35]. Prominent expression in the eyes and in the abundant cones was observed in these animals (Fig. 1). Transgene expression was first detected in the eyes beginning at stages 40–42 later than the endogenous gene [9], most likely because of the low sensitivity of fluorescence. GFP expression was variable between animals and reached its maximum about 5–8 days after nuclear transplantation. Fluorescence intensity noticeably faded after three to four weeks and was not detectable in post-metamorphic animals. In the first week, transgenic tadpoles exhibited GFP expression also in pineal (see below), even though the endogenous gene was not detectable by RT-PCR (Fig. 1C). Transient ectopic transgene expression was reported for other retinal promoters [17]. Most significantly, retinal expression was limited to cones (Fig. 1D) and GFP was found in all cellular compartments.

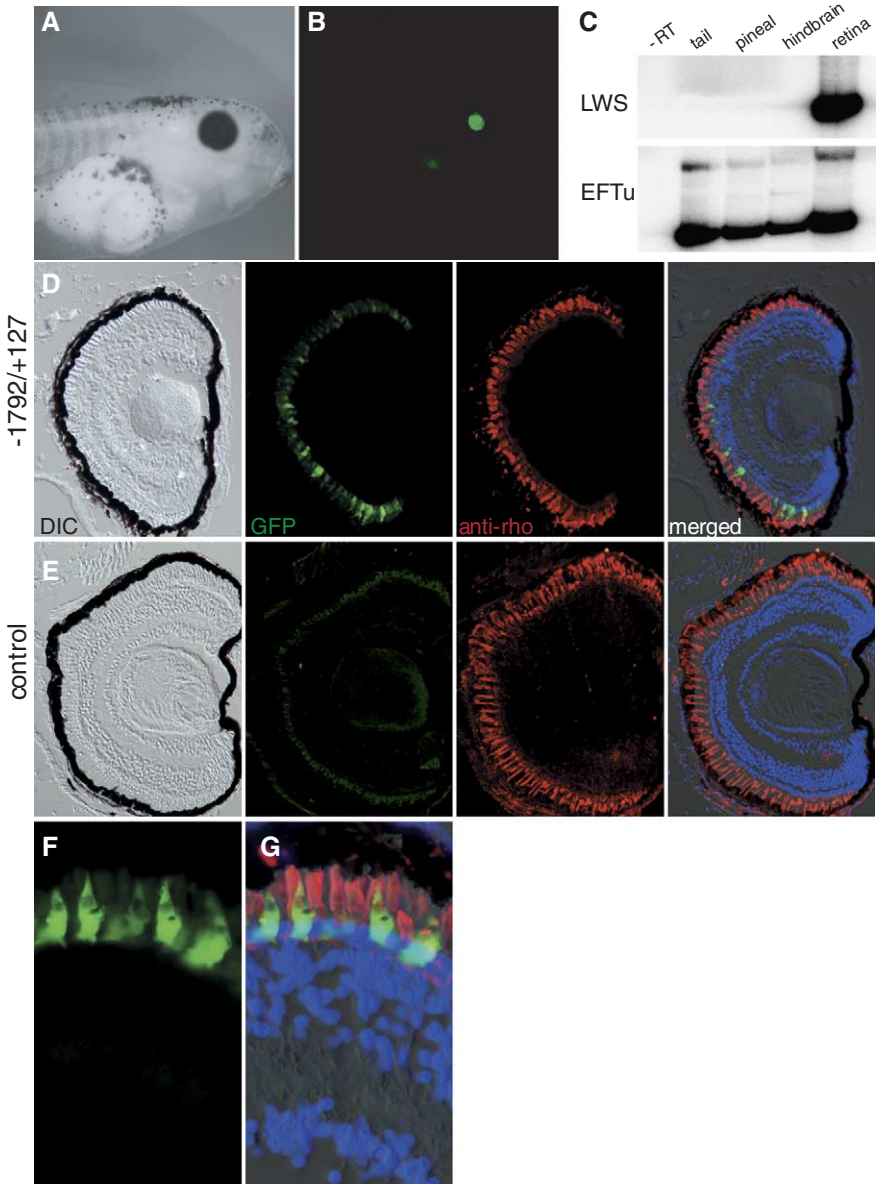

Fig. 1. 5′ flanking sequence (−1792/+127) of LWS opsin directs eye-specific transgene expression in *Xenopus* tadpoles. Brightfield (A) and fluorescent (B) images of a transgenic animal (stage 46) expressing GFP under control of the *Xenopus* LWS opsin promoter. (C) Reverse transcriptase-PCR analysis of transcripts from *Xenopus* tadpoles (stage 46). Oligo dT-primed cDNA was prepared from total RNA and PCR was performed using primers from either LWS exon 1 or EFTu as a loading control. (−RT), no reverse transcriptase. (D, E) 12 μm cross sections of control retinas (stage 46) stained with anti-rhodopsin antibody (red) and DAPI (blue). (F, G) Magnified image of GFP-expressing cone cells.

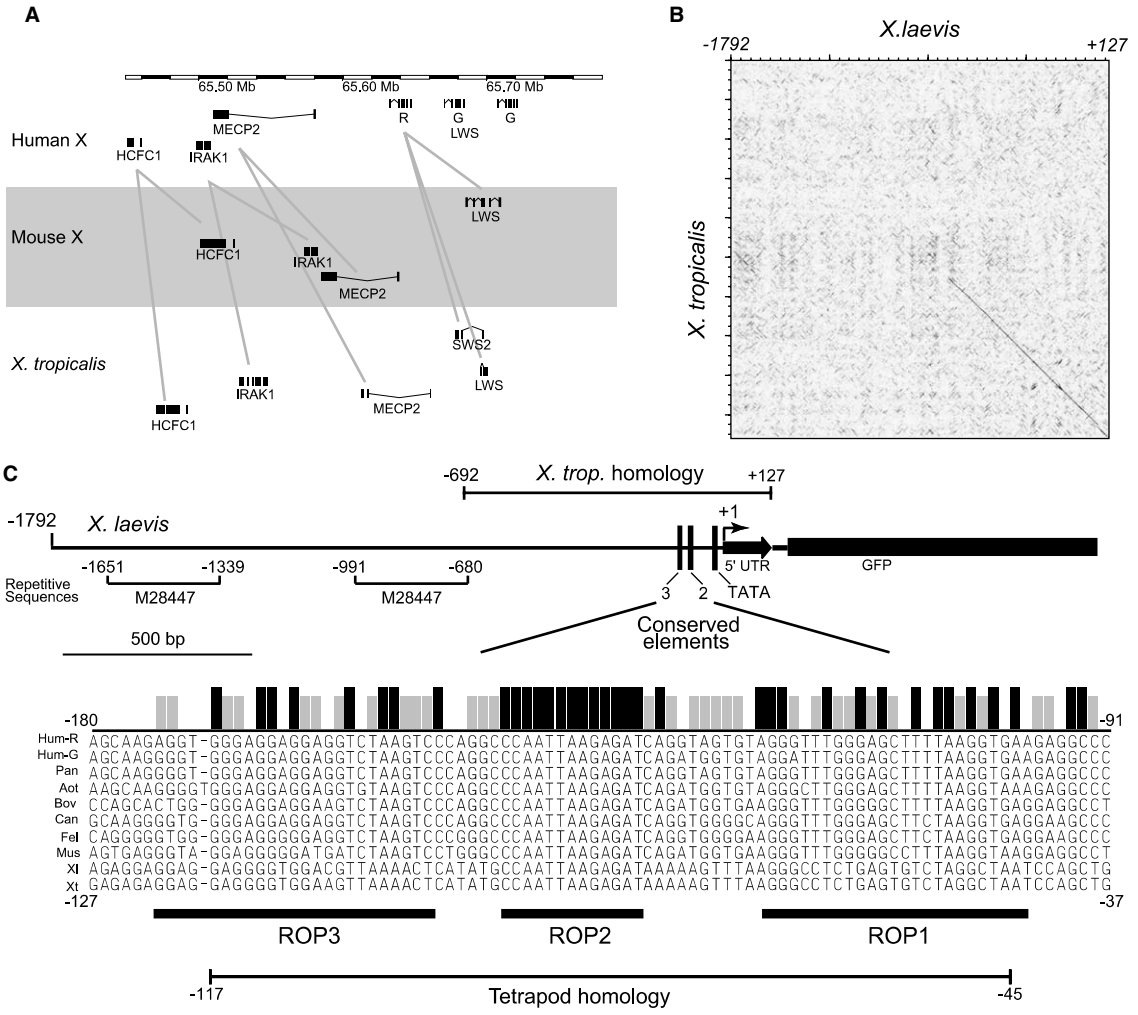

Fig. 2. Evolutionarily conserved chromosome region harboring LWS locus and sequence similarity in LWS proximal promoter region. (A) Structure of the human, mouse and *Xenopus* chromosomal regions harboring LWS locus. (B) Dot plot of 5′ upstream regions of *X. laevis* and *X. tropicalis* LWS genes. (C) Schematic of the LWS proximal promoter upstream of GFP with relative positions of frog and tetrapod homology domains. There are two copies of a repetitive sequence (*Xori*, accession number M28447 [43]). Sequence alignments of the immediate upstream nucleotides of *X. tropicalis* (Xt), *X. laevis* (Xl), cow (Bov), cat (Fel), dog (Can), mouse (Mus), owl monkey (Aot), chimpanzee (Pan), human green opsin (Hum-G), and human red opsin (Hum-R). Regions of 100% identity are indicated with black bars, those with 80% matches with grey. The numbers on top of the sequences represent the positions for human red and the numbers on the bottom of the sequences for *X. laevis*.

3.2. Conserved upstream sequences

We compared chromosomal regions containing M/LWS genes in vertebrates [37] with obvious synteny in these loci (Fig. 2A). Extensive similarities were found between closely related species. For example, the upstream sequences of pufferfish *Tetradon nigroviridis* and *Fugu rubripes* are 81% identical, while zebrafish is only 37–39% similar to either of the pufferfish sequences. *X. laevis* and *X. tropicalis* share 91% sequence identity in the immediate upstream region (Fig. 2B), which we refer to as the frog homology domain. The immediate 500 bp upstream regions had no recognizable similarity between fish and tetrapods. Phylogenetic footprinting [34] of tetrapod M/LWS genes revealed the presence of a highly conserved stretch of DNA located between −170 and −93 in the human promoter and in closer proximity to the TATA box in *X. laevis*, between −117 and −45 (Fig. 2C). We refer to this region as the tetrapod homology domain. Within this domain (Fig. 2C), there was a 13 base pair identity (ROP2) in all 10 tetrapods, 5′–CCAATTAAGAGAT-3′, containing two common motifs: CCAATT-binding factor (G*CCAAT*) and c-Myc (*TAAGAGA*). This sequence also has similarity to the Ret1 core conserved sequence (AAG*CCAATTA*) found in tetrapod rhodopsin promoters [16], but less conservation with a similar sequence in fish rhodopsin promoters [38]. A number of transcription factors, including CRX and QRX, bind to the rhodopsin Ret1 [39,40] red opsin promoter elements (ROP2) contains the core *AATTA* consensus site [41] and is likely to bind Q50 homeodomains (such as QRX) than a K50 homeodomain (such as CRX). Further evidence for a functional role of this region, including the moderately conserved GA-rich sequence (ROP3), was reported in DNAse footprinting experiments on the human red cone pigment using retinoblastoma nuclear extracts [28].

3.3. Control of tissue-specific transcription

We prepared a number of plasmids in which the 5′ upstream flanking region was partially deleted or disrupted. A large

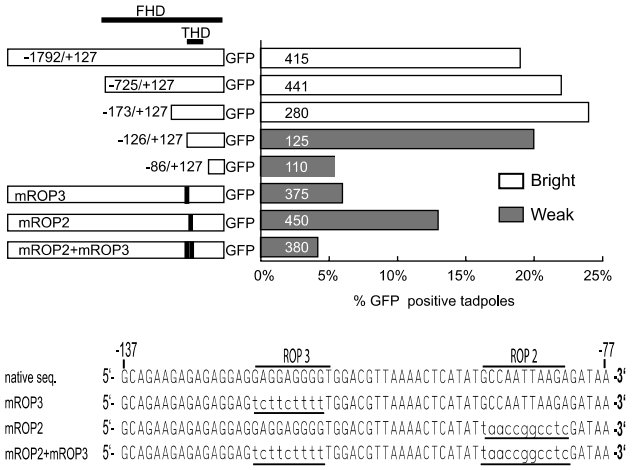

Fig. 3. Conserved 5′ proximal sequences of *Xenopus* LWS promoter are required for appropriate transgene expression. (*Top*) LWS promoter-GFP constructs used to produce transgenic animals are shown next to the total number and the percentages of GFP-positive tadpoles. The locations of frog homology domain (FHD) and tetrapod homology domain (THD) are indicated. The relative expression levels are indicated with white bars representing bright GFP expression and grey bars with weak/heterogeneous GFP expression. (*Bottom*) Sequences used to generate targeted mutations.

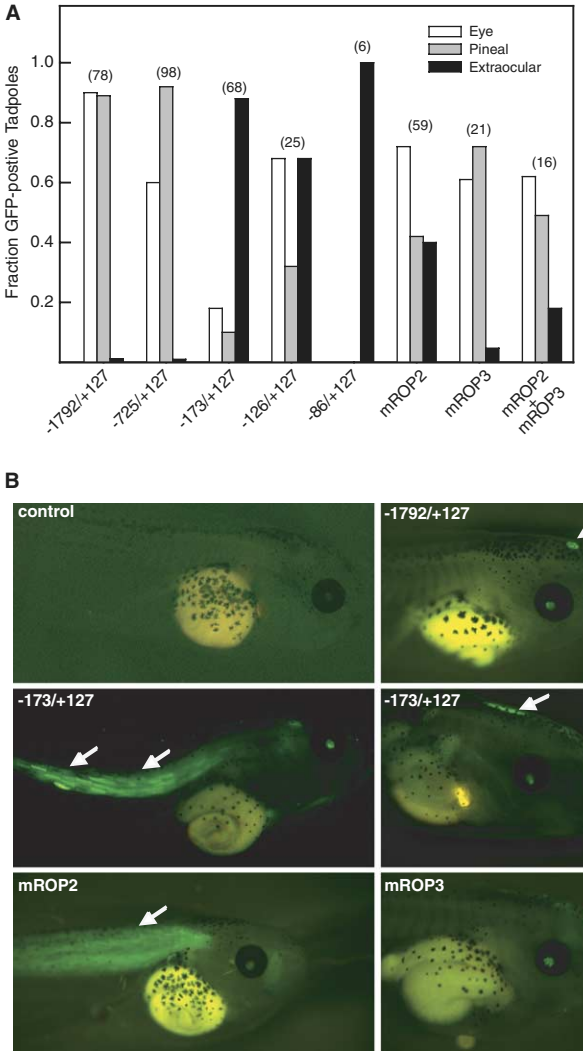

Fig. 4. 5′ proximal sequences of *Xenopus* LWS gene required for tissue-specific expression. (A) Tissue specificity of GFP expression was characterized in living tadpoles as percentage tadpoles with eye, pineal and extraocular expression (some animals expressed in more than one tissue). (B) Examples of transgenic animals with extraocular GFP expression. Arrows indicate (from top, counter clockwise) pineal, hindbrain, olfactory placode and surrounding tissue, muscles and spinal nerves.

number of tadpoles was examined to control for potential integration site or transgene copy number effects (Fig. 3). The tissue specificity was characterized in living tadpoles as eye, pineal, or extraocular, with some animals having expression in multiple tissues. Transgenic animals created using the longest constructs (−1792/+127 and −725/+127) exhibited bright ocular/pineal expression and a very low percentage (0.1%) of extraocular expression. We found that the shortest constructs (−128 and −86) and mutants in the tetrapod homology domain had greatly reduced expression (Fig. 3, shaded bars). Elimination of a large portion of the frog homology domain (−173/+127) resulted in dramatic loss of tissue specificity. Only 20% of the transgenic animals expressed GFP in the eye, while GFP expression was found in spine, hindbrain, heart, muscles and facial nerves (Fig. 4B). Deletion of the next 48 base pairs resulted in a similar GFP distribution accompanied by a great reduction in expression, and the shortest fragment (−86/+127) directed no detectable GFP in most animals. Mutation of ROP2 domain resulted in significant GFP expression in the spine, hindbrain, heart, jaw and muscles (Fig. 4B). Mutation of ROP3 had only a minor effect on tissue-specificity. Mutation of both ROP2 and ROP3 resulted in very weak GFP levels, near the autofluorescence limits.

3.4. Control of cone-specific transcription

Within the retina, the minimal construct (−725/+127) directed cone-specific expression in 10 day old tadpoles, a stage when cones are fully differentiated and before the gradual loss of expression in older animals (*see above*). This is consistent with a previous observation using a similar fragment (−769/+59) of the *Xenopus* LWS gene [33]. The (−173/+127) construct directed GFP expression not only in cones but also in most rods and some bipolar cells (Fig. 5A and E). Thus, the sequence between −725 and −173 contains one or more elements that restrict transgene expression to cones within the retina. A short construct missing a portion of the tetrapod homology domain (−126/+127) did not exhibit detectable GFP expression within the retina (*data not shown*), indicating a potential *cis*-element located within −173 and −126 required for transcriptional activation. Mutation of ROP3 did not alter the cone-specific expression of the transgene (Fig. 5B) suggesting that the ROP3 region was not involved in specifying cone expression within the retina. A mutation in ROP2 directed GFP expression not only in cones but also in inner and ganglion cell layers (Fig. 5C and D). This suggests that ROP2 region not only plays a role in restricting expression in extraocular tissues, but also plays a role in regulating cell-specific expression within the retina. The double mutation, ROP2–ROP3, eliminated detectable GFP expression from the eye. Thus, ROP2 and ROP3 elements may work together to regulate level of transcription as well as tissue specificity of the LWS gene.

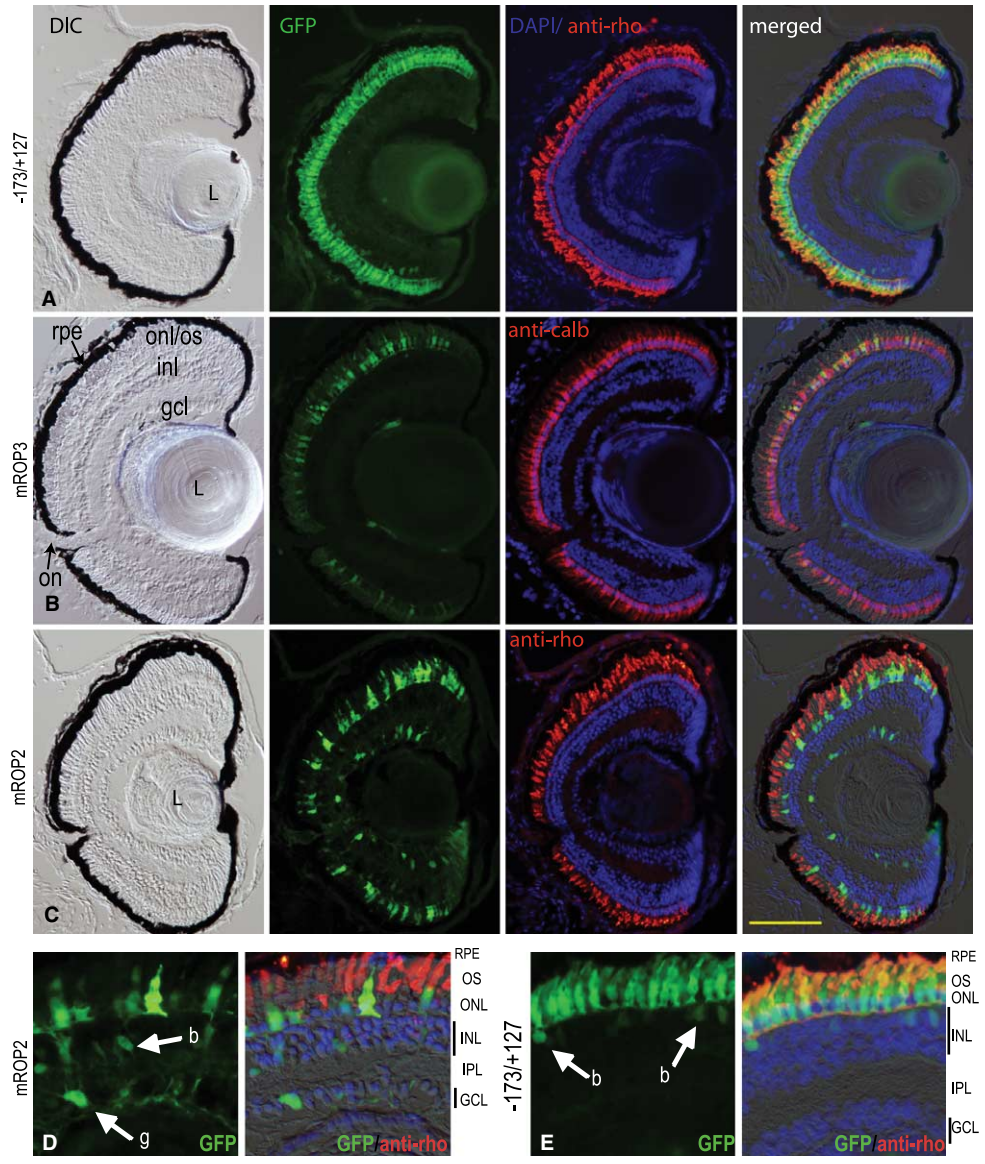

Fig. 5. Control of cell-specific expression by sequences within the *Xenopus* LWS opsin proximal promoter. (A) The −173/+128 construct directed transgene expression not only in cones, but also in rods and bipolar cells (also see E). (B) Mutation of ROP3 region did not detectably alter cone specific expression of GFP. (C) Mutation of 11 bp within the ROP2 conserved region caused expression to occur throughout inner nuclear and ganglion cell layers (also see D). (D, E) Higher magnification of retina produced with ROP2 mutant and (−173/+127) constructs. Scale bar = 100 μm. b, bipolar cell; g, ganglion cell; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; L, lens; ONL, outer nuclear layer; ON, optic nerve; OS, outer segment; RPE, retinal pigment epithelium.

4. Discussion

In this report, we have uncovered evolutionarily conserved tissue- and cell-restricting sequences in the M/LWS opsin promoter responsible for regulating cell-specific expression. DNA sequences from the tetrapod homology domain do not clearly match those for identified cone transcription factors, but there is a TAATC (OTX/Crx) motif (−47/−45, Fig. 2C), as indicated by Moritz et al., which is conserved in *Xenopus* but not in all tetrapods. In vitro transfection experiments in HEK293 cells show only ~6-fold stimulation by Crx and less with other known factors (*data not shown*). Experiments in zebrafish have uncovered novel regulatory mechanisms that control expression of SWS opsins [42]. Detailed analysis of M/LWS promoters has yet to be achieved. Transgenic *Xenopus* approaches have the potential to significantly accelerate the experimental analysis of transcriptional regulatory elements in tetrapod cone promoters, particularly in light of the apparent evolutionary changes that have occurred in opsin genes since the divergence between fish and tetrapods.

Acknowledgments: We acknowledge A. Eisner for technical assistance, E. Solessio for expert assistance with tadpole dissections and S. Mani, A. Viczian and M. Zuber for helpful advice. This work was supported by the National Institutes of Health Grants EY-11256 and EY-12975 (B.E.K.), Research to Prevent Blindness (Unrestricted Grant to SUNY UMU Department of Ophthalmology) and Lions of CNY.

References

[1] Okano, T., Kojima, D., Fukada, Y., Shichida, Y. and Yoshizawa, T. (1992) Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. Proc. Natl. Acad. Sci. USA 89, 5932–5936.

[2] Yokoyama, S. (1997) Molecular genetic basis of adaptive selection: examples from color vision in vertebrates. Annu. Rev. Genet. 31, 315–336.

[3] Yokoyama, S. (2000) Molecular evolution of vertebrate visual pigments. Prog. Retin. Eye Res. 19, 385–419.

[4] Nathans, J. (1999) The evolution and physiology of human color vision: insights from molecular genetic studies of visual pigments. Neuron 24, 299–312.

[5] Ebrey, T. and Koutalos, Y. (2001) Vertebrate photoreceptors. Prog. Retin. Eye Res. 20, 49–94.

[6] Roderick, R.W. (1998) The first steps in seeing. Sinauer Associates, Sunderland, Mass, p. 562.

[7] Morrow, E.M., Furukawa, T. and Cepko, C.L. (1998) Vertebrate photoreceptor cell development and disease. Trends Cell Biol. 8, 353–358.

[8] Stenkamp, D.L., Barthel, L.K. and Raymond, P.A. (1997) Spatiotemporal coordination of rod and cone photoreceptor differentiation in goldfish retina. J. Comp. Neurol. 382, 272–284.

[9] Chang, W.S. and Harris, W.A. (1998) Sequential genesis and determination of cone and rod photoreceptors in *Xenopus*. J. Neurobiol. 35, 227–244.

[10] Perron, M., Kanekar, S., Vetter, M.L. and Harris, W.A. (1998) The genetic sequence of retinal development in the ciliary margin of the *Xenopus* eye. Dev. Biol. 199, 185–200.

[11] Foster, R.G. and Hankins, M.W. (2002) Non-rod, non-cone photoreception in the vertebrates. Prog. Retin. Eye Res. 21, 507–527.

[12] Van Gelder, R.N., Herzog, E.D., Schwartz, W.J. and Taghert, P.H. (2003) Circadian rhythms: in the loop at last. Science 300, 1534–1535.

[13] Kumar, R. et al. (1996) The bzip transcription factor nrl stimulates rhodopsin promoter activity in primary retinal cell cultures. J. Biol. Chem. 271, 29612–29618.

[14] Whitaker, S.L. and Knox, B.E. (2004) Conserved transcriptional activators of the *Xenopus* rhodopsin gene. J. Biol. Chem. 279, 49010–49018.

[15] Chen, S. et al. (1997) Crx, a novel otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. Neuron 19, 1017–1030.

[16] Mani, S.S., Batni, S., Whitaker, L., Chen, S., Engbretson, G. and Knox, B.E. (2001) *Xenopus* rhodopsin promoter. Identification of immediate upstream sequences necessary for high level, rod-specific transcription. J. Biol. Chem. 276, 36557–36565.

[17] Knox, B.E., Schlueter, C., Sanger, B.M., Green, C.B. and Besharse, J.C. (1998) Transgene expression in *Xenopus* rods. FEBS Lett. 423, 117–121.

[18] Peng, G.H., Ahmad, O., Ahmad, F., Liu, J. and Chen, S. (2005) The photoreceptor-specific nuclear receptor nr2e3 interacts with crx and exerts opposing effects on the transcription of rod versus cone genes. Hum. Mol. Genet..

[19] Friedman, J.S. et al. (2004) The minimal transactivation domain of the basic motif-leucine zipper transcription factor nrl interacts with tata-binding protein. J. Biol. Chem. 279, 47233–47241.

[20] Mitton, K.P., Swain, P.K., Chen, S., Xu, S., Zack, D.J. and Swaroop, A. (2000) The leucine zipper of nrl interacts with the crx homeodomain. A possible mechanism of transcriptional synergy in rhodopsin regulation. J. Biol. Chem. 275, 29794–29799.

[21] Cheng, H., Khanna, H., Oh, E.C., Hicks, D., Mitton, K.P. and Swaroop, A. (2004) Photoreceptor specific nuclear receptor nr2e3 functions as a transcriptional activator in rod photoreceptors. Hum. Mol. Genet..

[22] Mears, A.J., Kondo, M., Swain, P.K., Takada, Y., Bush, R.A., Saunders, T.L., Sieving, P.A. and Swaroop, A. (2001) Nrl is required for rod photoreceptor development. Nat. Genet. 29, 447–452.

[23] Zhang, J. et al. (2004) Rb regulates proliferation and rod photoreceptor development in the mouse retina. Nat. Genet. 36, 351–360.

[24] Viczian, A.S., Verardo, M., Zuber, M.E., Knox, B.E. and Farber, D.B. (2004) Conserved transcriptional regulation of a cone phototransduction gene in vertebrates. FEBS Lett. 577, 259–264.

[25] Wang, Y., Smallwood, P.M., Cowan, M., Blesh, D., Lawler, A. and Nathans, J. (1999) Mutually exclusive expression of human red and green visual pigment-reporter transgenes occurs at high frequency in murine cone photoreceptors. Proc. Natl. Acad. Sci. USA 96, 5251–5256.

[26] Smallwood, P.M., Wang, Y. and Nathans, J. (2002) Role of a locus control region in the mutually exclusive expression of human red and green cone pigment genes. Proc. Natl. Acad. Sci. USA 99, 1008–1011.

[27] Wang, Y., Macke, J.P., Merbs, S.L., Zack, D.J., Klaunberg, B., Bennett, J., Gearhart, J. and Nathans, J. (1992) A locus control region adjacent to the human red and green visual pigment genes. Neuron 9, 429–440.

[28] Shaaban, S.A. and Deeb, S.S. (1998) Functional analysis of the promoters of the human red and green visual pigment genes. Invest. Ophthalmol. Vis. Sci. 39, 885–896.

[29] Boatright, J.H., Knox, B.E., Jones, K.M., Stodulkova, E., Nguyen, H.T., Padove, S.A., Borst, D.E. and Nickerson, J.M. (2001) Evidence of a tissue-restricting DNA regulatory element in the mouse irbp promoter. FEBS Lett. 504, 27–30.

[30] Mani, S.S., Besharse, J.C. and Knox, B.E. (1999) Immediate upstream sequence of arrestin directs rod-specific expression in *Xenopus*. J. Biol. Chem. 274, 15590–15597.

[31] Zhu, X., Ma, B., Babu, S., Murage, J., Knox, B.E. and Craft, C.M. (2002) Mouse cone arrestin gene characterization: promoter targets expression to cone photoreceptors. FEBS Lett. 524, 116–122.

[32] Lerner, L.E., Gribanova, Y.E., Ji, M., Knox, B.E. and Farber, D.B. (2001) Nrl and sp nuclear proteins mediate transcription of rod-specific cgmp-phosphodiesterase beta-subunit gene: involvement of multiple response elements. J. Biol. Chem. 276, 34999–35007.

[33] Moritz, O.L., Peck, A. and Tam, B.M. (2002) *Xenopus laevis* red cone opsin and prph2 promoters allow transgene expression in amphibian cones, or both rods and cones. Gene 298, 173–182.

[34] Barta, E., Sebestyen, E., Palfy, T.B., Toth, G., Ortutay, C.P. and Patthy, L. (2005) Doop: Databases of orthologous promoters, collections of clusters of orthologous upstream sequences from chordates and plants. Nucleic Acids Res. 33, D86–D90.

[35] Amaya, E. and Kroll, K.L. (1999) A method for generating transgenic frog embryos. Methods Mol. Biol. 97, 393–414.

[36] Molday, R.S. and MacKenzie, D. (1983) Monoclonal antibodies to rhodopsin: characterization, cross-reactivity, and application as structural probes. Biochemistry 22, 653–660.

[37] Hubbard, T. et al. (2005) Ensembl 2005. Nucleic Acids Res. 33, D447–D453.

[38] Zhang, T., Tan, Y.H., Fu, J., Lui, D., Ning, Y., Jirik, F.R., Brenner, S. and Venkatesh, B. (2003) The regulation of retina specific expression of rhodopsin gene in vertebrates. Gene 313, 189–200.

[39] Wang, Q.L. et al. (2004) Qrx, a novel homeobox gene, modulates photoreceptor gene expression. Hum. Mol. Genet. 13, 1025–1040.

[40] Morabito, M.A., Yu, X. and Barnstable, C.J. (1991) Characterization of developmentally regulated and retina-specific nuclear protein binding to a site in the upstream region of the rat opsin gene. J. Biol. Chem. 266, 9667–9672.

[41] Qian, J., Esumi, N., Chen, Y., Wang, Q., Chowers, I. and Zack, D.J. (2005) Identification of regulatory targets of tissue-specific transcription factors: application to retina-specific gene regulation. Nucleic Acids Res. 33, 3479–3491.

[42] Luo, W., Williams, J., Smallwood, P.M., Touchman, J.W., Roman, L.M. and Nathans, J. (2004) Proximal and distal sequences control uv cone pigment gene expression in transgenic zebrafish. J. Biol. Chem. 279, 19286–19293.

[43] Riggs, C.D. and Taylor, J.H. (1987) Sequence organization and developmentally regulated transcription of a family of repetitive DNA sequences of *Xenopus laevis*. Nucleic Acids Res. 15, 9551–9565.