BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **250,** 175–180 (1998)
ARTICLE NO. RC989261

Erx, a Novel Retina-Specific Homeodomain Transcription Factor, Can Interact with Ret 1/PCEI Sites

Julian A. Martinez* and Colin J. Barnstable†,[1]
†*Department of Ophthalmology and Visual Science and* **Interdepartmental Neuroscience Program, Yale University School of Medicine, New Haven, Connecticut 06520*

Received August 4, 1998

Our previous studies on the transcriptional regulation of rod opsin gene expression had defined a strikingly conserved element, Ret 1/PCEI, present in the upstream regulatory regions of opsin and other photoreceptor-specific genes. This element interacts with a 40 kDa, developmentally regulated, retina-specific protein. In this study we report the cloning of the novel retina-specific homeodomain protein Erx. Erx contains a homeodomain that is 79% homologous to that of *Drosophila* empty spiracles. This 40 kDa protein can interact with the Ret 1 element in electrophoretic mobility shift assays. Mutation of key residues in Ret 1 eliminates all Erx binding. Transient transfection of Y79 retinobalstoma cells with Erx leads to significant transcriptional activation of a reporter gene via Ret 1 elements. We conclude that Erx is the Ret 1 binding activity. This is the first example of a Q50 homeodomain protein expressed in retinal photoreceptors. © 1998 Academic Press

Key Words: **homeodomain; retina; photoreceptor; transcription factor.**

The rat rhodopsin gene qualifies as a good model for studies of cell type-specific differentiation pathways for several reasons. First, rod opsin, a member of the superfamily of G-protein coupled seven transmembrane spanning receptors, is found exclusively in one cell type in the retina, rod photoreceptors (1,2). Second, rod photoreceptors are one of the final cell types to differentiate in the postnatal retina and opsin is one of the first genes expressed by these cells. This makes photoreceptors an accessible cell type for study and opsin a well defined marker early in their differentiation. Third, previous studies show that this onset of opsin expression occurs by means of transcriptional activation (3,4). Clearly, isolation of those protein factors involve din regulating the early events of cell-type-specifc expression of opsin would further expand our understaning of photoreceptor differentiation. One method to achieve this goal involves the study of varying lengths of upstream promoter regulatory sequences of the rod opsin gene in transgenic animals. This approach narrows down those regions involve din DNA-protein interactions essential for the restriction of opsin expression to photoreceptors. One study showed that 500 bp upstream of the mouse opsin gene are sufficient to drive lacZ reporter gene expression in photoreceptors of transgenic mice, albeit with inappropriate temporal and spatial expression (5). A shorter 230 bp mouse opsin promoter is sufficient to reproduce retinal degeneration in vivo by targeting the expression of the SV40 T antigen exclusively to photoreceptors (6). Similar results can be obtained with a stretch of 200bp of bovine opsin promoter, which is sufficient to drive photoreceptor-specific lacZ expression, although not uniformly across the retina (7). These studies demonstrate that the promoter sequences which regulate photoreceptor-specific expression of opsin are functionally conserved from mice to humans. It is not surprising then that alignment of all cloned upstream sequences of rod opsin genes from several species reveals this proximal 200bp region to be the most strikingly conserved at the sequence level.

Experimental analysis using this 200 bp region from the bovine and rat opsin proximal promoters has allowed for the characterization of several protein-binding DNA elements involved in interactions with retinal proteins (8). Previous experiments in our laboratory localized one of the tissue-specific sequences to a 27bp binding site at position −125 to −110 of the rat opsin promoter, which we termed Ret 1. DNAse I footprinting analysis of this region revealed protection only with adult retinal nuclear protein extrats, and gel retardation assays confirmed the presence of protein binding in a temporal pattern overlapping that of opsin upregulation (9). Within this Ret1 binding site, a series of residues has

[1] Address correspondence to Dr. Colin J. Barnstable, Department of Ophthalmology and Visual Science, Yale University School of Medicine, 330 Cedar Street, P.O. Box 208061, New Haven, CT 06520-8061. Fax: (203) 785-7401. E-mail: colin.barnstable@yale.edu.

0006-291X/98 $25.00

been conserved across vertebrate and invertebrate species for opsin-like genes (10; 11) as well as for several photoreceptor-specific genes, including S-antigen/arrestin (12; 13), guanylate cyclase-E (14), gamma-transducin (15), the beta subunit of cGMP-phosphodiesterase (16), hydroxyindole-O-methyltransferase (17), and interstitial retinol-binding protein (IRBP: 18,19). This consensus sequence, CAATTAG, is able to compete for binding to the Ret 1 site in gel shift assay while point mutations of specific bases abolishes in vitro Ret 1 binding activity (9). Southwestern analysis reveals that the Ret 1 binding protein is a 40 kDa protein whose expression is upregulated by FGF (20). In a recent report we presented evidence that RET1 is both necessary and sufficient to drive the photoreceptor-specific expression of a reporter gene *in vivo* (21). Interestingly, the conserved sequence present in this element shares homology with the consensus binding site for a subclass of homeodomain-containing proteins (9).

In order to determine whether the rat rod opsin gene is a downstream target of homeodomain proteins in photoreceptors, we used the rod opsin promoter in an assay for isolating members of this class of transcription factors. In this study we describe the characterization of a novel retina-specific transcription factor we have named Erx (empty-spiracles-related retinal homeobox). We demonstrate that Erx is a novel member of the empty spiracles subfamily of homeodomain transcription factors. Its cDNA encodes a protein of 40 kDa which can interact specifically with the Ret 1 element. It is exclusively present in the developing retina during stages of opsin expression and localizes to differentiated photoreceptor cells in the adult. We therefore conclude that Erx represents a likely candidate gene for encoding the Ret1 binding activity.

MATERIALS AND METHODS

Yeast one-hybrid screen. Our screen was performed as per manufacturer's instructions (Clontech) using an adult rat retina cDNA library (1.5×10^6 independent clones) in pGAD10 vector (Clontech).

RT-PCR and EMSA. Both RT-PCR and electrophoretic mobility shift assays (EMSA) were performed as previously described (21). For the BAT-1 experiments, oligonucleotides were synthesized corresponding to the region -62 to -81 of the rat opsin promoter. The binding buffer used for BAT-1 EMSA was previously described (8).

In vitro translation. We prepared full length capped sense transcripts for Erx by using standard methods provided in the MaxiScript Kit (Ambion). These transcripts were run in formaldehyde gels to verify their size and integrity. In vitro translation was performed using a rabbit reticulocyte lysate as per manufacturer's instructions (Promega).

Transient transfection. Y79 retinoblastoma cells were plated the day before transfection at 2×10^5 per 60 mm petri dish. Transfection was performed by the calcium phosphate method as previously described (22). Two days after transfection cells were fixed for 5 minutes at room temperature in 4% paraformaldehyde. Assays for β-galactosidase activity were performed as previously described (21). The fullest length Erx clone was digested as an EcoRI fragment and cloned into the EcoRI site of the pcDNA3 vector using standard methods. The lacZ vector (Ret 1)x4-hsp68-*lac*Zpa used was prepared as previously described (21).

RESULTS

Isolation of DNA-Binding Factors That Interact with the Ret 1 Element

In order to identify factors which may regulate opsin expression we used four copies of the Ret 1 element in the yeast one-hybrid system. After screening $>3 \times 10^6$ clones of an adult rat retina library, we isolated several colonies which demonstrated specific binding to the Ret 1 element multimer. These colonies carried plasmids which encode for novel proteins as well as previously identified factors. The results of this screen will be described elsewhere. A series of clones encoded transcripts of different lengths for the same novel protein. The longest transcript consisted of 1.2 kb and contained both a short poly-A+ tail as well as the most 5′ end of any of the transcripts isolated. WE expected this transcript to encode the full-length protein since we have the poly-A+ sequence in its extreme 3′ end and a Kozak sequence adjacent to the most upstream ATG in its 5′ end. After comparing the nucleotide sequence to the NCBI database, we identified this transcript as homologous to the divergent *Drosophila* homeobox gene *empty spiracles (ems;* 23). Sequence comparison within the translated homeodomain reveals 78% similarity with empty spiracles and 72% with the mamalian homologues Emx1 and Emx2 (Figure 1). We therefore named this transcript Erx, for empty spiracles-related retinal homeobox. The amino acid sequence demonstrates the presence of a glutamine at residue 50 of the homeodomain (Q50), amino acid 9 of the recognition helix, helix 3/4. This is characteristic of the *Antennapaedia* subclass of homeodomain proteins and differentiates it from others which contain a Lysine (*bicoid*/*orthodenticle*/*sine oculis*), cysteine (LIM-homeodomain proteins), or serine (Oct proteins). A retinal human EST homologue was already present in the databse at the time of cloning (Accession #: H92142).

Erx Is a Retina-Specific Transcript Present in Adult Photoreceptors

In our previous studies, we had identified regions of Ret 1 binding activity in vivo by placing four copies of this element in front of a *lac*Z reporter gene (21). We detected expression in adult retinal photoreceptors and pineal gland, both known sites of rhodopsin expression in mice (24). This was consistent with biochemical data which had defined the Ret 1 binding activity as a retina-specific, developmentally regulated complex (9). We determined the tissue distribution pattern of Erx expression by performing RT-PCR reactions on cDNA

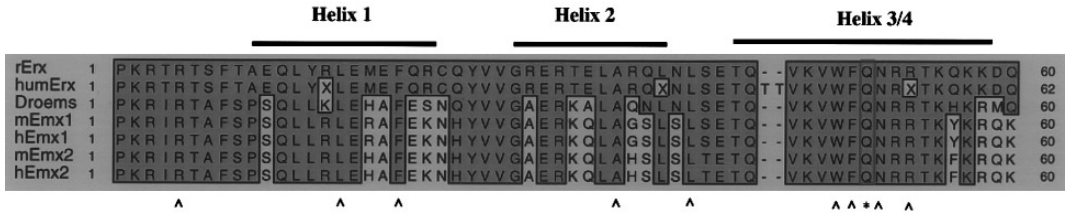

FIG. 1. Alignment of Erx homeodomain with other members of the *empty spiracles* subfamily. Boxed residues represent homologous sequences. Arrowheads point at invariant residues conserved in all homeodomains. An asterisk demarcates the glutamine present at position 9 of helix 3/4, the recognition helix. Human Erx was obtained from translation of EST#H92142. r, rat; m, mouse; h, human.

from different adult tissues. Erx was only identified in the adult mouse retina (Figure 2A). It was not present in adult human RPE, ciliary body, cerebellum, cerebral cortex, spleen, lung, heart, kidney, or liver.

To determine if Erx expression is dependent on the presence of photoreceptors in the adult retina, we performed RT-PCR on cDNA from 6 month old C3H mice. This strain carries a mutation in the rd gene which causes complete degeneration of rod photoreceptor cells by 6 months of age (25). No specific Erx product was detected, while actin, a marker for successful reverse transcription was detected (Figure 2B). We conclude that Erx is exclusively expressed by photoreceptor cells in the retina.

Erx Encodes for a 40 kDa Sequence-Specific DNA-Binding Protein that Interacts with the Ret 1 Element

The molecular weight of Erx was determined by in vitro translating capped in vitro transcripts of the 1.2kb rat cDNA clone. Analysis of reticulocyte lysate samples by SDS-PAGE reveals a slowest migrating band of approximately 40 kDa (Figure 3A). Other minor forms of lower molecular weight are present, probably due to multiple starts of translation. This molecular weight is very similar to that of the predicted Ret1 protein (9). We thus conclude that the Erx transcripts encodes a protein of 40 kDa.

The ability of this in vitro translated protein to interact with the Ret1 element was tested using electrophoretic mobility shift assays. Incubation of adult retinal extracts with oligonucleotides of the Ret 1 element leads to formation of a retina-specific complex. A single complex of similar migration is observed with in vitro translated Erx. This complex can be eliminated by incubation of a mutated Ret 1 oligonucleotide with Erx or adult retina protein extract (Figure 3B). These mutations have been shown to disrupt Ret 1 element activity both in vitro (9) and in vivo (21). To verify its binding specificity, we incubated Erx protein with an oligo for the BAT-1 element, which forms a complex distinct from Ret 1 in adult retinal extracts. No complex was detected with Erx and BAT-1 (Figure 3B). Our data confirm that Erx behaves in vitro in a manner identical to that of the Ret 1 binding protein of retina.

Erx Can Upregulate Transcription via Ret 1 Elements in Transient Transfection

To define the ability of Erx to investigate gene expression via Ret 1 elements, we used a previously described vector (21) in transient transfection assays of Y79 retinoblastoma cells. We were unable to detect any *lac*Z+ cells after a transient transfection of the hsp68-*lac*ZpA vector alone (Figure 4). When we transiently transfected four copies of the Ret 1 element in front of the *lac*Z gene, a small number of *lac*Z+ cells were observed. However, co-transfection of the (Ret 1) x4-hsp68-*lac*ZpA and an Erx expression vectors yields a significant six-fold increase in the number of *lac*Z+ cells (Figure 4; $p<0.002$; student's t-test). This demonstrates that Erx can activate transcription via Ret 1 elements. However, transient transfection of Erx into Y79 cells does not lead to ectopic expression of the endogenous rod opsin gene (data not shown).

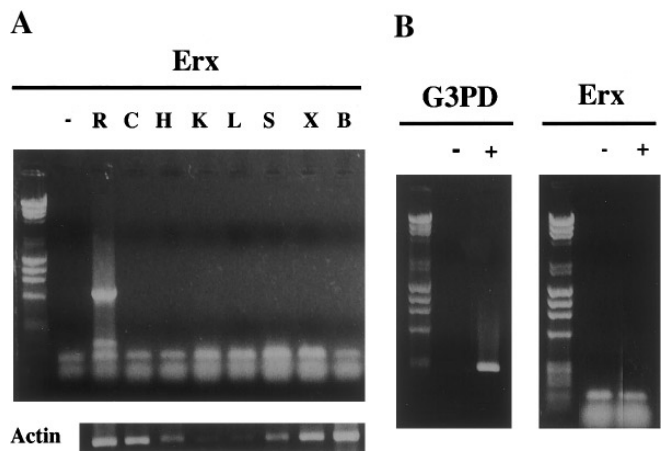

FIG. 2. A. Retina-specific pattern of Erx expression in adult mouse tissues by RT-PCR. R, retina; C, cerebellum; H, heart, K, kidney; L, liver; S, spleen; X, cerebral cortex, B, brainstem. B. Absence of Erx transcripts in >6 months old C3H retinas as determined by RT-PCR. Minus/plus signs indicate absence/presence of cDNA respectively. G3PD, glyceraldehyde 3-phosphate dehydrogenase.

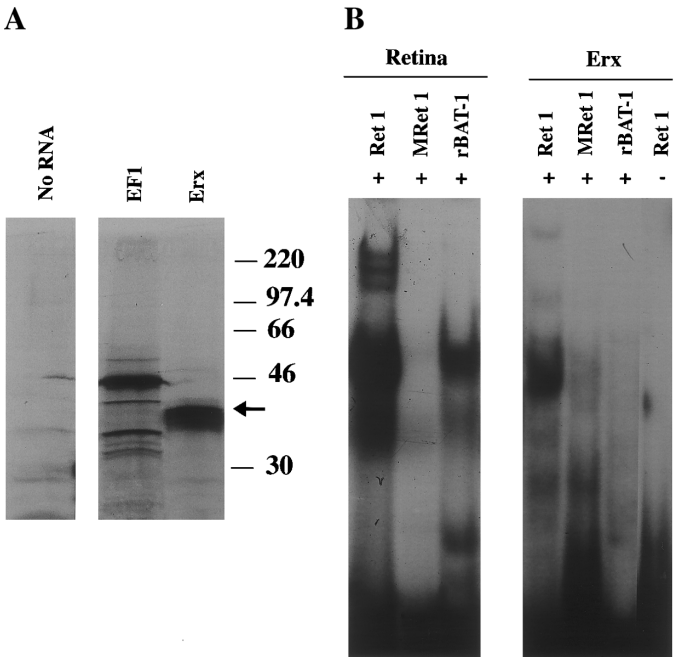

FIG. 3. A. In vitro translation of Erx coding sequence. Arrow points to largest polypeptide with a molecular weight of 40 kDa. EF1 RNA was used as a positive control for translation. B. Electrophoretic mobility shift assay of Erx binding activity. Adult CD-1 retinal protein extracts (10 μg/lane) incubated with radiolabeled oligonucleotides for either Ret 1, MRet 1, or rBAT-1 elements were used in the three lanes on the left. In vitro translated Erx protein was used in the three lanes to the right. Minus/plus signs indicate absence/presence of protein respectively.

DISCUSSION

Our initial studies on the transcriptional regulation of the rat rod opsin promoter yielded several retina-specific binding activities within the proximal 200 bp upstream of the start of transcription. These included the Ret 2 element (26), BAT-1 element (27), and the Ret 1 element (9). Our present study has used the yeast one-hybrid system to identify proteins which can interact with the Ret 1 element, a 27 base pair sequence present in the proximal promoter of the rod opsin gene (9). To determine if we had indeed isolated a candidate transcript for the Ret 1 binding activity several characteristics had to be confirmed. These have been previously described in our laboratory and include: 1, the Ret 1 complex is retina-specific (9); 2, the binding activity is present in a single polypeptide of 40 kDa from late embryonic to adult stages in the developing retina (20); 3, it interacts specifically with the Ret 1 element and this interaction can be disrupted by mutations of specific bases in its consensus sequence; and 4, its positive regulatory activity is present in adult retinal photoreceptors (21). In this study we have characterized Erx, a novel retina-specific member of the family of homeodomain-containing proteins. Erx expression is present in postnatal retina (Martinez and Barnstable, unpublished results) and is maintained in mature photoreceptors, consistent with a role in formation and maintenance of rods. In addition, this protein behaves biochemically in a manner identical to the Ret 1 binding activity. We conclude therefore that we have identified the gene which encodes for the Ret 1 binding protein and classified it as a member of the *empty spiracles*-related subfamily of homeodomain transcription factors (28).

Homeodomain proteins are characterized by the presence of a 60 aa helix-turn-helix protein motif, the homeodomain, which interacts with DNA in a sequence-specific manner through specific contacts within a highly invariant signature recognition helix (29). Initially discovered in Drosophila, their role in pattern formation and cell fate determination has been conserved across phyla (30, 31). The number of described members of the homeodomain superfamily has exploded in recent years (32), with each new example further affirming their central role in organismal development (33). In the eye, the individual players and transcriptional networks in which homeodomain factors fall are starting to become clear (34, 35, 36). However, despite the fact that several classes of homeodomain subfamilies have been shown to have a role in eye development, very few examples exist of the target genes they recognize in individual cell types. Our data demonstrates that the rod opsin gene is a target of homeodomain proteins through interactions with the Ret 1 element.

Ret1/PCEI elements are not only present in the promoters of opsin genes from vertebrate species but in all *Drosophila* opsin gene regulatory regions as well (13, 37). In *Drosophila,* Pax-6 may have a role in opsin

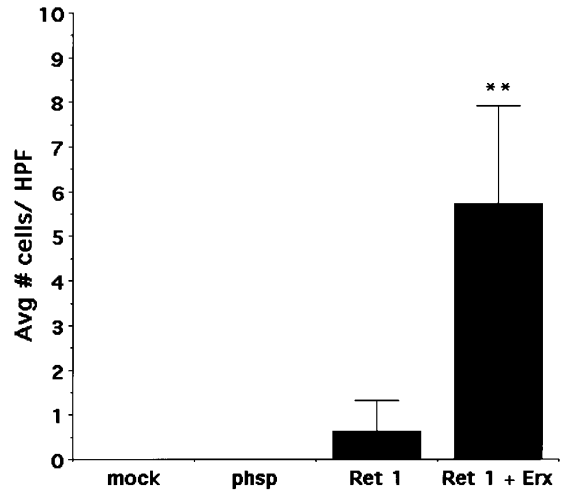

FIG. 4. Transient transfection of Y79 retinoblastoma cells. Significance ($p<0.002$, student's t-test) is represented by asterisks, phsp, hsp68-*lac*Zp A vector; Ret 1, (Ret 1) x4-hsp68-*lac*ZpA vector; Erx, full coding sequence in pcDNA3 expression vector.

expression via binding to these sites (38). It can bind to Ret 1 elements located upstream of the Rh1 opsin gene, the closest homologue to vertebrate rod opsin of all the *Drosophila* opsins. This appears consistent with evidence demonstrating that Pax-6 can induce ectopic eyes, and therefore appears to be a master regulator of eye development (39). However the role of this transcription factor in vertebrate eye development appears to be different (40, 41, 42). There are some lines of evidence which suggest a role for Pax-6 in photoreceptor development. Additional copes of the Pax-6 gene can lead to poorly differentiated or the complete absence of photoreceptors (43). In lower invertebrates, Pax-6 is expressed in photoreceptive sensory organs (44). However, while ectopic Pax-6 expression in Xenopus leads to ectopic lens formation (45), Hirsch and Harris concluded that Pax-6 alone may not have a role in inducing ectopic eyes or in photoreceptor development (46). Expression patterns in the developing retina do not localize Pax-6 to photoreceptors at any stage in development (47, 48). What is clear is that both Erx and Pax-6 share a glutamine at positions 50 of the homeodomain, which may account for their similar functions in binding to Ret 1/PECI sites in the promoters of photoreceptor-specific genes.

Despite the unclear evolutionary role of Pax-6 in regulation of photoreceptor genes, a pattern of conserved promoter regulation does emerge from studies of opsin gene promoter function in *Drosophila*. It is perhaps a common theme in gene regulation that there will be two elements within the proximal promoter of photoreceptor genes: one for binding of proteins with a lysine at residue 50 of the homeodomain (K50 proteins) and one for proteins with a glutamine at the same position (Q50 proteins). These may act synergistically in the proximal promoter and their heteromeric interaction may provide increased specificty of expression (49). We known that Crx, a photoreceptor-specific K50 homeodomain protein, can interact with GGATTA elements, characteristic of proteins of this subclass (50, 51). It is possible that its close homolouge *orthodenticle*, which is required for late stages of photoreceptor development, binds to the K50 site present in the opsin promoters of *Drosophila* (52). On the other hand, Pax-6, a Q50 homeodomain protein, could interact with its high affinity CAATTA sites in photoreceptor genes of Drosophila as Erx does in vertebrates. The close physical proximity of these elements and their proteins suggest a mode of action for tissue-specific opsin expression in which heterodimeers of Q50/K50 homeodomain proteins may form. Erx and Crx probably interact with each other as heterodimers while Crx interacts with Nrl (51), a retina-specific basic lecuine zipper DNA-binding factor involved in opsin expression (53). The absence of opsin expression in Y79 cells, even in the presence of Nrl, Erx, and Crx (Martinez and Barnstable, unpublished results), strongly suggests the possibility that other factors are missing or that strong negative regulation occurs via selective binding of a repressor to the opsin promoter. This chain of interactions could synergize in the proximal promoter and lead to the cell-type specific, synchronous activation of photoreceptor genes in the postnatal retina.

It is clear from our findings that several tissue-specific binding activities come together in the opsin enhancer/promoter to activate photoreceptor-specific expression of this gene. We anticipate that many more DNA-binding factors as well as cofactors, both tissue-speciifc and not, will add to the complex interactions that must be required for the cell-type specific expression of the rod opsin gene in photoreceptors. An understanding of these regulatory mechanisms of expression, both temporal and tissue-specific, will be necessary for identifying possible targets to heritable retinal degenerative diseases and for find-tuned genetic therapeutic approaches.

ACKNOWLEDGMENTS

We thank all members of the Barnstable Lab past and present for their help and valuable conversations. We thank Dr. Juan Sanchez-Torres for guidance in molecular biology protocols, Dr. Kenneth Wikler for critical reading of the manuscript, and Stephen Viviano and Adrienne LaRue for outstanding technical assistance. This work is part of the requirements for a thesis in the INP at Yale University. This work was supported by grants NS20483, and The Foundation Fighting Blindness to CJB. JAM was supported by a predoctoral NRSA EY06558.

REFERENCES

1. Barnstable, C. J., Blum, A. S., Devoto, S. H., Hicks, D., Morabito, M. A., Sparrow, J. R., and Treisman, J. E. (1988) *Neurosci. Res.* Suppl. **8,** 527–41.
2. Barnstable, C. J., and Morabito, M. A. (1994) *J. Mol. Neurosci.* **5,** 207–209.
3. Treisman, J. E., Morabito, M A., and Barnstable, C. J. (1988) *Mol. & Cell. Biol.* **8,** 1570–1579.
4. DeJardin, L. E., Timmers, A. M., and Hauswirth, W. W. (1993). *J. Biol. Chem.* **268,** 6953–6960.
5. Lem, J., Applebury, M. L., Falk, J. D., Flannery, J. G., and Simon, M. I. (1991) *Neuron* **6,** 201–210.
6. al-Ubaidi, M. R., Hollyfield, J. G., Overbeek, P. A., and Baehr, W. (1992) *Proc. Natl. Acad. Sci. U S A* **89,** 1194–1198.
7. Zack, D. J., Bennett, J., Wang, Y., Davenport, C., Klaunberg, B., Gearhart, J., and Nathans, J. (1991) *Neuron* **6,** 187–199.
8. DesJardin, L. E., and Hauswirth, W. W. (1996) *Invest. Ophthalmol. Vis. Sci.* **37,** 154–165.
9. Morabito, M. A., Yu, X., and Barnstable, C. J. (1991) *J. Biol. Chem.* **266,** 9667–9672.
10. Fortini, M., and Rubin, G. M. (1990) *Genes Dev.* **4,** 444–463.
11. Sheshberadaran, H., and Takahashi, J. S. (1994) *Mol. Cell. Neurosci.* **5,** 309–318.
12. Krishnan, R., and Gnaguly, R. (1995) *Gene* **160,** 185–195.
13. Kikuchi, T., Raju, K., Breitman, M. L., and Shinihara, T. (1993) *Mol. Cell. Biol.* **13,** 4400–4408.
14. Johnston, J. P., Farhangfar, F., Aparicio, J. G., and Nam, S. H., and Applebury, M. L. (1997) *Gene* **197,** 219–227.

15. Tao, L., Pandey, S., Simon, M. I., and Fong, H. K. W. (1993) *Exp. Eye Res.* **56,** 497–507.
16. DiPolo, A., Rickman, C. B., and Farber, D. B. (1996) *Invest. Ophthalmol. Vis. Sci.* **37,** 551–560.
17. Rodriguez, I. R., Mazuruk, K., Schoen, T. J., and Chader, G. J. (1994) *J. Biol. Chem.* **269,** 31969–31977.
18. Liou, G. I., Matragoon, S., Yang, J., Geng, L., Overbeek, P. A., and Ma, D. P. (1991) *Biochem. Biophys. Res. Commun.* **181,** 159–165.
19. Liou, G. I., Matragoon, S., Overbeek, P. A., and Fei, Y. (1994) *Biochem. Biophys. Res. Commun.* **203,** 1875–1881.
20. Yu, X., and Barnstable, C. J. (1995) *J. Mol. Neurosci.* **5,** 259–271.
21. Yu, X., Leconte, L., Martinez, J. A., and Barnstable, C. J. (1996) *J. Neurochem.* **67,** 2494–2504.
22. Chen, C. I., and Okayama, H. (1987) *Mol. Cell. Biol.* **7,** 2745–2752.
23. Walldorf, U., and Gehring, W. J. (1992) *E.M.B.O. J.* **11,** 2247–2259.
24. Blackshaw, S., and Snyder, S. H. (1997) *J. Neurosci.* **17,** 8074–8082.
25. Garcia-Fernandez, J. M., Jimenez, A. J., and Foster, R. G. (1995) *Neurosci. Lett.* **187,** 33–36.
26. Yu, X., Chung, M., Morabito, M. A., and Barnstable, C. J. (1993) *Biochem. Biophys. Res. Commun.* **191,** 76–82.
27. Martinez, J. A., and Barnstable, C. J. (1997) *Mol. Biol. Cell.* **8,** 225a.
28. Simeone, A., Gulisano, M., Acampora, D., Stornaiuolo, A., Rambaldi, M., and Boncinelli, E. (1992) *E.M.B.O. J.* **11,** 2541–2550.
29. Gehring, W. J., Affolter, M., and Burglin, T. (1994) *Ann. Rev. Biochem.* **63,** 487–526.
30. Lawrence, P. A., and Morata, G. (1994) *Cell* **78,** 181–189.
31. Manak, J. R., and Scott, M. P. (1994) *Dev.* **Suppl,** 61–77.
32. Stein, S., Fritsch, R., Lemaire, L., and Kessel, M. (1996) *Mech. Dev.* **55,** 91–108.
33. Mark, M., Rijli, F. M., and Chambon, P (1997) *Pediatr. Res.* **42,** 421–429.
34. Oliver, G., and Gruss, P. (1997) *Trends Neurosci.* **20,** 415–421.
35. Freund, C., Horsford, D. J., and McInnes, R. R. (1996) *Hum. Mol. Gen.* **5,** 1471–1488.
36. Beebe, D. C. (1994) *Invest. Ophthalmol. Vis. Sci.* **35,** 2897–2900.
37. Wilson, D., Guenther, B., Desplan, C., and Kuriyan, J. (1995) *Cell* **82,** 709–719.
38. Sheng, G., Thouvenot, E., Schmucker, D., Wilson, D. S., and Desplan, C. (1997) *Genes Dev.* **11,** 1122–1131.
39. Halder, G., Callaerts, P., and Gehring, W. J. (1995) *Science* **267,** 1788–1792.
40. Macdonald, R., and Wilson, S. W. (1996) *Curr. Opin. Neurobiol.* **6,** 49–56.
41. Fernald, R. D. (1997) *Brain Behav. Evol.* **50,** 253–259.
42. Harris, W. A. (1997) *Proc. Natl. Acad. Sic. U S A* **94,** 2098–2100.
43. Schedl, A., Ross, A., Lee, M., Engelkamp, D., Rashbass, P., van Heyningen, V., and Hastie, N. D. (1996) *Cell* **86,** 71–82.
44. Callaerts, P., Halder, G., and Gehring, W. J. (1997) *Annu. Rev. Neurosci.* **20,** 483–532.
45. Altmann, C. R., Chow, R. L., Lang, R. A., and Hemmati-Brivanlou, A. (1997) *Dev. Biol.* **185,** 119–123.
46. Hirsch, N., and Harris, W. A. (1997) *J. Neurobiol.* **32,** 45–61.
47. Davis, J. A., and Reed, R. R. (1996) *J. Neurosci.* **16,** 5082–94.
48. Belecky-Adams, T., Tomarev, S., Li, H. S., Ploder, L., McInnes, R. R., Sundin, O., and Adler, R. (1997) *Invest. Ophthalmol. Vis. Sci.* **38,** 1293–1303.
49. Han, K., Levine, M. S., and Manley, J. L. (1989) *Cell* **56,** 573–583.
50. Furukawa, T., Morrow, E. M., and Cepko, C. L. (1997) *Cell* **91,** 531–541.
51. Chen, S., Wang, Q. L., Nie, Z., Sun, H., Lennon, G., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., and Zack, D. J. (1997) *Neuron* **19,** 1017–1030.
52. Vandendries, E. R., Johnson, D., and Reinke, R. (1996) *Dev. Biol.* **173,** 243–255.
53. Rehemtulla, A., Warwar, R., Kumar, R., Ji, X., Zack, D. J., Swaroop, A. (1996) *Proc. Natl. Acad. Sci. U S A* **93,** 191–195.