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Genetics

The basic motif–leucine zipper transcription factor Nrl can positively regulate rhodopsin gene expression

(retinal genes/retinal diseases/*maf* oncogene family/DNA-binding protein)

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ABSTRACT **The retinal protein Nrl belongs to a distinct subfamily of basic motif–leucine zipper DNA-binding proteins and has been shown to bind extended AP-1-like sequence elements as a homo- or heterodimer. Here, we demonstrate that Nrl can positively regulate the expression of the photoreceptor cell-specific gene rhodopsin. Electrophoretic mobility-shift analysis reveals that a protein(s) in nuclear extracts from bovine retina and the Y79 human retinoblastoma cell line binds to a conserved Nrl response element (NRE) in the upstream promoter region of the rhodopsin gene. Nrl or an antigenically similar protein is shown to be part of the bound protein complex by supershift experiments using Nrl-specific antiserum. Cotransfection studies using an Nrl-expression plasmid and a luciferase reporter gene demonstrate that interaction of the Nrl protein with the −61 to −84 region of the rhodopsin promoter (which includes the NRE) stimulates expression of the reporter gene in CV-1 monkey kidney cells. This Nrl-mediated transactivation is specifically inhibited by coexpression of a naturally occurring truncated form of Nrl (dominant negative effect). Involvement of Nrl in photoreceptor gene regulation and its continued high levels of expression in the adult retina suggest that Nrl plays a significant role in controlling retinal function.**

The ontogeny of retinal cell types during development has been established by anatomical and cell biological studies combined with thymidine birth-dating and lineage-tracing techniques (1–4). However, the molecular mechanisms governing the generation of photoreceptors and other neuronal cells in the mammalian retina are poorly understood. Since the induction of cell type-specific genes is involved in the commitment of multipotent precursor cells to specific neuronal lineages (5), a direct approach for dissecting the molecular events during retinal differentiation is to elucidate the mechanisms regulating differential gene expression in the retina and define the components of the transcriptional regulatory hierarchy. Identification of transcription factors and their target genes in specific retinal cell types should help to delineate the pathways of cell fate determination and may provide insights into the pathological mechanisms responsible for congenital and inherited diseases affecting retinal function.

Several transcription factor genes including homeobox (Hox), POU, paired box (Pax), and basic motif–leucine zipper (bZIP) sequences are expressed during eye development (6–11). However, their function in controlling developmental stage- and/or cell type-specific gene expression in the retina has not been determined. One of these potential regulatory genes, *NRL* (neural retina leucine zipper), cloned by a subtractive hybridization approach (11–13), encodes a member of the bZIP family of DNA-binding proteins (14). It is highly conserved between mouse and man and demonstrates a retina-specific pattern of expression on Northern analysis (11, 13). The Nrl protein shows strong sequence homology to the v-*maf* oncogene product (15). Maf and Nrl define a distinct subfamily of bZIP proteins that includes the c-*maf* protooncogene product (16), several other Maf proteins (17, 18), the small subunit p18 of the erythroid transcription factor NF-E2 (19), and the product of the segmentation gene kriesler (*kr*) (20). Nrl can form homodimers; it also can heterodimerize with Maf and with several members of the c-Fos and c-Jun family (21, 22). The Nrl homo- and heterodimers bind to the consensus sequence $TGCN_{6-8}GCA$ (Nrl response element, NRE), where the internal core (N_{6-8}) is similar to the AP-1 binding site (TGACTCA) or cAMP response element (TGAGCTCA) (21, 22). The presence of an NRE-like sequence in the promoter region of the photoreceptor-specific rhodopsin gene prompted us to explore whether the rhodopsin gene is a target for regulation by Nrl. Here we report that Nrl is part of the retinal nuclear protein complex which binds to the rhodopsin NRE and demonstrate that it can transactivate reporter gene expression by interacting with rhodopsin promoter sequences which include the NRE. Nrl-mediated transactivation is specifically inhibited by a naturally occurring truncated Nrl protein lacking 105 aa in the N-terminal region. Our results suggest that Nrl is involved in the regulation of rhodopsin gene expression.

MATERIALS AND METHODS

Nuclear Extracts and Antisera. Nuclear extracts from bovine retina, liver, and kidney were prepared as described (23, 24). The HeLa and Y79 retinoblastoma cell nuclear extracts, as well as the c-Fos and c-Jun antibodies (TransCruz gel supershift reagent), were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The α2.3-Nrl antiserum, prepared against full-length bacterially expressed Nrl, and the corresponding preimmune serum were provided by Tom Kerppola (22). The P1 antiserum was generated against an 11-aa synthetic peptide derived from the proline/serine/threonine (P/S/T)-rich acidic region of Nrl (aa 23–33; see ref. 11) by Berkeley Antibody (Richmond, CA). The specificity of α2.3 and P1 antisera was confirmed by immunoprecipitation experiments using mock and Nrl-transfected COS cell extracts.

Electrophoretic Mobility-Shift Analysis (EMSA). The oligonucleotides RhoP and mRhoP (Fig. 1*A*), were synthesized at the University of Michigan Biomedical Research Core

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Abbreviations: bZIP, basic motif–leucine zipper; EMSA, electrophoretic mobility-shift analysis; NRE, Nrl response element; t-Nrl, truncated Nrl.

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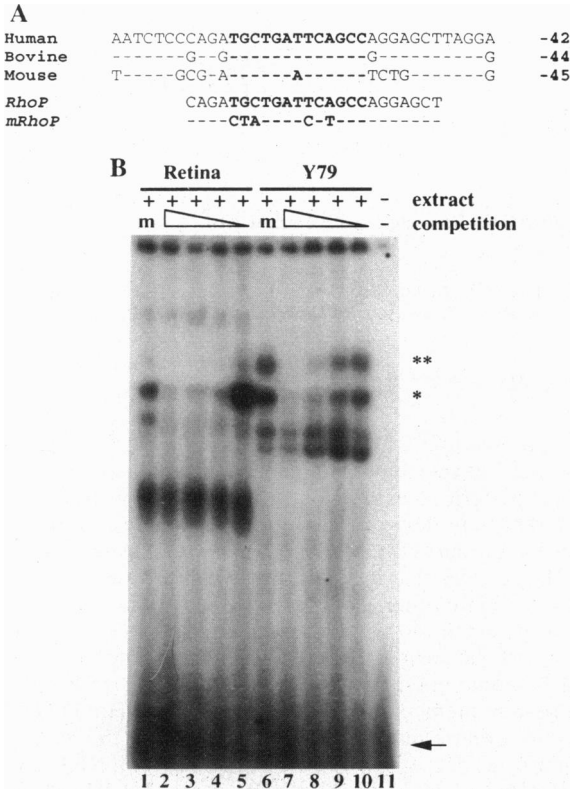

FIG. 1. EMSA using the rhodopsin NRE. (*A*) Conservation of the putative NRE (sequence shown in bold) in the promoter region upstream of the human, bovine, and mouse rhodopsin genes (25). Only nucleotides that diverge from human are shown in the bovine and mouse sequence. The sequences of RhoP and mRhoP oligonucleotides used for EMSA are also shown. (*B*) Binding of the rhodopsin NRE to proteins in bovine retinal and Y79 retinoblastoma nuclear extracts. The ^{32}P-labeled double-stranded RhoP oligonucleotide was used for EMSA with retinal (lanes 1–5) or Y79 (lanes 6–10) extract. Competition with unlabeled oligonucleotides was performed to determine the specificity of binding. Lanes 5 and 10, no unlabeled competing oligonucleotide; lanes 4–2 and 9–7, increasing concentration (5, 100, and 500-fold molar excess, respectively) of unlabeled RhoP; lanes 1 and 6, 500-fold molar excess of the mutant mRhoP oligonucleotide. The sample in lane 11 did not include any protein. The experiments were performed at least four different times. Formation of two of the shifted RhoP bands (* and **) is efficiently inhibited with excess unlabeled RhoP but not with mRhoP and represents specific bound nuclear protein complexes. Arrow indicates the position of free oligonucleotide.

Facility. EMSA was performed essentially as described (26). One of the RhoP oligonucleotides was end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP and annealed to the complementary unlabeled oligomer. The double-stranded oligonucleotide (0.2–1 ng, $\approx 10^5$ cpm) was incubated in binding buffer with nuclear extract (5–30 μg) and poly(dI-dC) (50 μg/ml) for 30 min at room temperature. In competition experiments, unlabeled double-stranded RhoP and mRhoP oligonucleotide was used. For supershift experiments, antibody or control serum was added after the incubation of ^{32}P-labeled RhoP with nuclear extracts. The samples were loaded on a prerun 5–8% polyacrylamide gel. After electrophoresis, the gel was dried and exposed to x-ray film for autoradiography.

Recombinant Plasmids. The human NRL (clone 321B1) and DD10 cDNAs (11) were cloned into the pMT3 vector (27) by using the *Eco*RI and *Sal* I restriction sites to generate plasmid constructs for expression in mammalian cells. The pMT-NRL plasmid expresses the wild-type Nrl protein, including a P/S/T-rich acidic region and a basic(+) motif followed by the leucine zipper domain. The pMT-DD10 plasmid encodes a truncated protein (t-Nrl) with an in-frame deletion of 105 aa (residues 23–127) including the N-terminal P/S/T-rich region. t-Nrl should be able to form homodimers, heterodimerize with the wild-type Nrl, and bind to DNA but is predicted to lack the transactivation function. The pMT-NRLΔZ plasmid was derived from pMT-NRL by deleting the sequence (bp 761–812 in the NRL cDNA; ref. 11) encoding part of the leucine zipper domain. Therefore, the resultant mutant protein lacks the dimerization and DNA-binding function of wild-type Nrl. Luciferase reporter constructs were generated from the pGL2-Basic vector (Promega, Madison, WI) by cloning various upstream regions of the rhodopsin gene (28). The p61-Luc, p84-Luc, p130-Luc, p176-Luc, and p225-Luc constructs contain sequences derived from the bovine rhodopsin gene extending from −61 to +72, −84 to +72, −130 to +72, −176 to +72, and −225 to +72 bp, respectively. The p4907-Luc construct contains mouse rhodopsin gene sequence extending from bp −4907 to bp +85.

Transfection Experiments. All transfections for the analysis of transcriptional activity of the wild-type and variant Nrl proteins were performed in CV-1 monkey kidney cells by the DEAE-dextran method (29) and included the pCMV-β-galactosidase expression plasmid as an internal control to normalize for variations in transfection efficiency. The activity of luciferase and β-galactosidase was measured in cell extracts, prepared 60 hr posttransfection, by the methods provided with the respective kits (Promega).

Analysis of Nrl Proteins Expressed in Transfected Cells. Expression of Nrl and its variant proteins from the pMT constructs was analyzed by published methods (29). Briefly, cesium chloride-purified DNA (8 μg) was transfected into $\approx 10^5$ COS-1 cells by the DEAE-dextran method. About 60 hr after transfection, cells were preincubated with methionine-free medium for 10 min before the addition of [^{35}S]methionine (1000 Ci/mmol) (Promix, Amersham) for 1 hr. Cells were washed in cold phosphate-buffered saline, and lysed in modified RIPA buffer (10 mM TrisCl, pH 7.5/1 mM EDTA/150 mM NaCl/0.5% Triton X-100/0.5% sodium deoxycholate/0.02% sodium azide) at 4°C for 15–30 min. The nuclear fraction was removed by centrifugation, and the supernatant was incubated overnight at 4°C with control preimmune serum or Nrl-specific antiserum (α2.3 or P1). The immunocomplex was isolated by incubation with protein A-Sepharose (Pharmacia). After washing in RIPA buffer, the samples were boiled in Laemmli buffer and analyzed by SDS/PAGE (10% or 14%) followed by fluorography.

RESULTS

Retinal Nuclear Extract Contains a Protein(s) That Interacts with the Rhodopsin NRE. Previous studies identified both proximal and distal regulatory regions upstream of the bovine and mouse rhodopsin genes (24, 25, 28, 30, 31). The proximal regulatory region at bp −225 to +72 in the bovine gene is sufficient to direct photoreceptor cell-specific expression of a reporter gene in transgenic mice, whereas the distal regulatory region (located ≈2 kb upstream) functions as an enhancer. The proximal upstream region contains several potential sites for DNA-binding proteins (28, 32–34), including an NRE-like sequence [bp −66 to −54 in the human sequence (35); Fig. 1*A*], which has been shown to bind to *Escherichia coli*-expressed Nrl (36). This putative NRE in the proximal regulatory region is highly conserved in the human, bovine, and mouse rhodopsin genes (Fig. 1*A*). EMSA of a 24-bp oligonucleotide, RhoP (Fig. 1*A*), corresponding to the human sequence containing the putative NRE, demonstrated specific shifts in mobility (indicated by * and **) upon incubation with bovine retinal nuclear extract (Fig. 1B, lane 5). The intensity of the shifted bands decreased with addition of increasing

amounts of unlabeled RhoP oligonucleotide (lanes 4–2), but no significant competition was observed even with a 500-fold molar excess of a mutant oligomer (mRhoP) containing five nucleotide substitutions in the NRE (lane 1). EMSA using the labeled mRhoP oligonucleotide detected no significant mobility shift with the retinal nuclear extract (data not shown).

Nuclear extract from the human Y79 retinoblastoma cell line (which expresses the *NRL* gene; ref. 11) showed mobility shifts with wild-type RhoP that were similar to those observed with the bovine retinal nuclear extract (Fig. 1*B*, lane 10). Unlabeled RhoP (lanes 9–7), but not mRhoP (lane 6), competed efficiently with the RhoP probe for formation of the shifted bands (* and ** in Fig. 1*B*). Nuclear extracts from cells and tissues that do not express *NRL*, such as HeLa cells, liver, and kidney (refs. 11 and 13; A.S., unpublished data), did not show evidence of specific gel shifts (data not shown).

Nrl Is Part of the Protein Complex That Binds to the Rhodopsin NRE. Addition of Nrl-specific antiserum α2.3 after incubation of RhoP with the retinal (Fig. 2, lane 3) or Y79 (lane 8) nuclear extract showed a mobility supershift (**) with a concomitant disappearance of the previously shifted complex (*). However, the addition of preimmune serum (lanes 4 and 9) did not supershift the RhoP complex. These results suggest that Nrl or an antigenically similar protein in the retinal and Y79 nuclear extracts is part of the protein complex that binds to the RhoP oligonucleotide. No significant supershift was observed with the antibodies against c-Jun (lanes 1 and 6) and c-Fos (lanes 2 and 7) under the present conditions, despite the ability of c-Jun and c-Fos to heterodimerize with Nrl (22).

Coexpression of Nrl Transactivates the Rhodopsin Promoter. To test the ability of Nrl to regulate rhodopsin gene expression in a heterologous cell culture system, we constructed three Nrl expression plasmids (Fig. 3*A*). pMT-NRL expressed in COS-1 cells yielded two major Nrl immunoreactive polypeptides of 43 and 35 kDa (Fig. 3*B*, lane 2), which were absent in the immunoprecipitate of mock-transfected cell

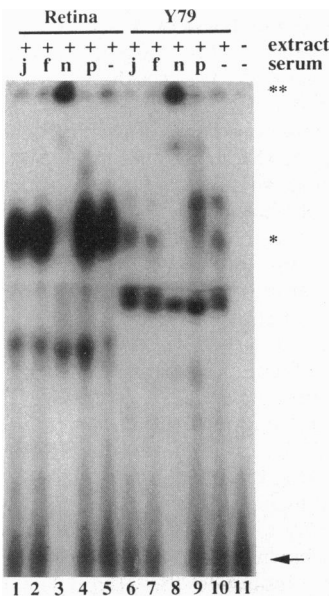

FIG. 2. The protein complex bound to the rhodopsin NRE contains Nrl or an antigenically related protein. The ^{32}P-labeled RhoP oligonucleotide was incubated with nuclear extracts derived from bovine retina (lanes 1–5) or Y79 retinoblastoma cells (lanes 6–10), followed by either preimmune serum (p) (lanes 4 and 9) or antiserum against c-Jun (j) (1 and 6), c-Fos (f) (lanes 2 and 7), or Nrl (n) (lanes 3 and 8). Lanes 5 and 10, RhoP with nuclear extract but no serum; lane 11, free RhoP with no nuclear extract. *, Position of shifted RhoP band(s); **, supershifted band; arrow, free oligonucleotide. The experiments were performed at least four times.

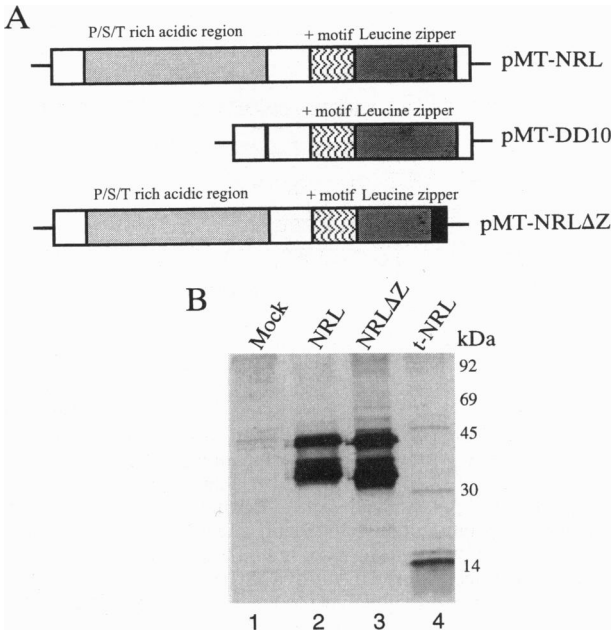

FIG. 3. Expression of Nrl proteins in mammalian cells. (*A*) Schematic representation of the Nrl proteins produced by the expression plasmids used. pMT-NRL expresses the wild-type Nrl protein, whereas pMT-DD10 encodes t-Nrl, lacking the N-terminal P/S/T-rich acidic region. The pMT-NRLΔZ plasmid expresses a mutant Nrl protein that lacks part of the leucine zipper and contains 18 unrelated amino acid residues at its C terminus (black box). (*B*) SDS/PAGE analysis of the immunoprecipitated wild-type and variant Nrl proteins. COS-1 cells were mock-transfected (lane 1) or transfected with pMT-NRL (lane 2), pMT-NRLΔZ (lane 3), or pMT-DD10 (lane 4).

extracts (lane 1). pMT-NRLΔZ, which has a deletion in the leucine zipper domain, yielded two Nrl immunoreactive bands of 41 and 33 kDa (Fig. 3*B*, lane 3). pMT-DD10, containing a previously identified truncated cDNA (DD10; ref. 11), produced polypeptides of 17 and 16 kDa (Fig. 3*B*, lane 4). The discrepancy between the predicted (31 kDa for the wild-type Nrl) and observed molecular masses of expressed Nrl protein appears to be due to differential posttranslational modification, as confirmed by metabolic labeling experiments in COS cells (A.R. and A.S., unpublished data). Western analysis of bovine and human retinal proteins detected similar high molecular weight polypeptides with affinity-purified Nrl-specific antibodies (X.J. & A.S., unpublished data). The size difference between the wild-type and variant proteins is in agreement with differences in their primary sequence.

To directly test the ability of Nrl to regulate rhodopsin expression, pMT-NRL was cotransfected into CV-1 cells with reporter constructs containing various fragments of the rhodopsin promoter. Cotransfection of pMT-NRL with the p84-Luc construct, which contains a complete NRE, consistently led to 3-fold stimulation of luciferase activity (Fig. 4). p61-Luc, which does not contain a complete NRE, showed no induction upon Nrl expression. p130-Luc and p176-Luc also showed responsiveness to Nrl, whereas p225-Luc and p4907-Luc were unresponsive to Nrl-mediated transactivation. Deletion of 41 bp in the multiple cloning site of the vector in p176-Luc (called pΔ176-Luc), which altered the junction region upstream of the promoter sequence, did not have any significant effect on Nrl-mediated transactivation (data not shown).

Truncated Nrl Inhibits Nrl-Mediated Transactivation. To examine the specificity of Nrl-mediated transactivation, pMT-NRLΔZ and pMT-DD10, encoding variant Nrl proteins (see above and Fig. 3), were tested in cotransfection experiments. These two plasmids had no effect on luciferase expression from the p84-Luc construct (Fig. 5). pMT-NRLΔZ, when

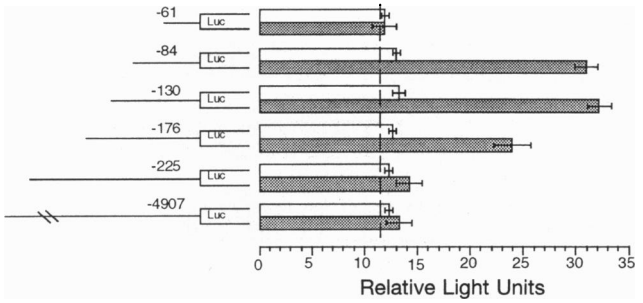

FIG. 4. Nrl-mediated transactivation of the rhodopsin promoter. The p4907-Luc, p225-Luc, p176-Luc, p130-Luc, p84-Luc, and p61-Luc (shown as -4907, -225, -176, -130, -84, and -61, respectively) constructs were transfected into CV-1 cells in the presence or absence of pMT-NRL. Cell extracts were assayed for luciferase and β-galactosidase activity. Data shown represent luciferase activity from three independent transfections (error bars represent 1 SD) and have been normalized using β-galactosidase activity that is driven by the cytomegalovirus promoter and hence not affected by Nrl expression. Vertical dashed line, background luminescence in the absence of added cell extract. Transactivation with p84-Luc and p130-Luc is statistically significant at $P \leq 0.0005$, and that with p176-Luc, at $P = 0.0015$.

cotransfected along with pMT-NRL, had no significant effect on the p84-Luc expression induced by Nrl; however, pMT-DD10 consistently inhibited Nrl-mediated transactivation of rhodopsin promoter activity (Fig. 5).

DISCUSSION

Several members of the Maf–Nrl subfamily of bZIP proteins have been identified and implicated in the regulation of cell type-specific gene expression (11, 13, 15–20). p18 and several small Maf proteins can dimerize with the p45 subunit of the NF-E2 transcription factor and regulate erythroid-specific

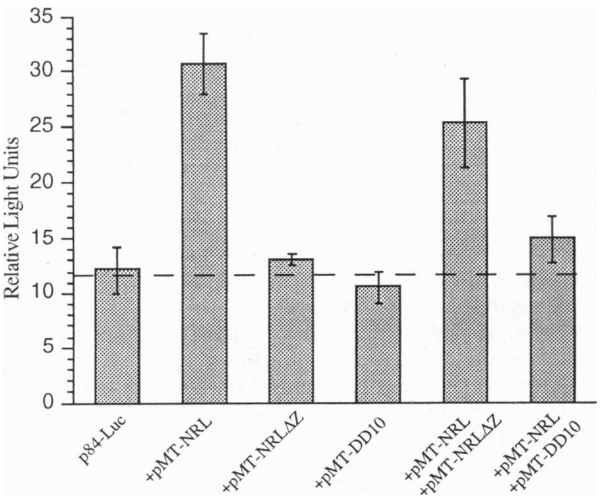

FIG. 5. Dominant negative effect of t-Nrl on Nrl-mediated transactivation. The p84-Luc reporter construct was transfected into CV-1 cells alone or in the presence of various Nrl expression plasmids (as indicated). Cell extracts were prepared and assayed for luciferase activity to determine Nrl-specific transactivation. β-Galactosidase activity was used to normalize for variations in transfection efficiency. Data represent luciferase activity from two independent transfections (error bars represent 1 SD). Similar results were obtained in three additional experiments performed with pΔ176-Luc. Horizontal dashed line, background luminescence in the absence of added cell extract. The dominant negative inhibition of Nrl-mediated transactivation with the pMT-DD10 construct is statistically significant at $P = 0.009$.

gene expression (19, 37, 38). c-Maf can stimulate transcription from a Purkinje neuron-specific promoter (16). The kreisler (*kr*) gene appears to be involved in hindbrain and otic development (20). Here, we report that the Nrl protein, which binds to an extended AP-1 element, participates in regulating the photoreceptor cell-specific gene rhodopsin.

Our studies directly implicate Nrl in mammalian photoreceptor cell-specific gene regulation. The presence of binding sites for several DNA-binding proteins in the upstream rhodopsin promoter region (28, 32–34) suggests that a combinatorial and synergistic interaction of multiple transcription factors (5, 39) is probably responsible for achieving stringently controlled cell type- and developmental stage-specific expression of the rhodopsin gene. We demonstrate by EMSA that a conserved NRE-like sequence in the proximal regulatory region of the rhodopsin promoter binds to Nrl (or an antigenically similar protein) present in the bovine retinal nuclear extract. Our results, however, do not reveal the nature of additional proteins that may be part of the protein complex bound to the rhodopsin NRE. The lack of supershift of RhoP complexes (obtained with either the retinal or Y79 extracts) by c-Jun or c-Fos antiserum suggests that the c-Jun and c-Fos proteins may not play a significant role in NRE-mediated regulation of rhodopsin expression.

Cotransfection studies in CV-1 cells, which do not express rhodopsin or *NRL*, show that the wild-type Nrl protein can interact with sequences in the rhodopsin promoter constructs p84-Luc, p130-Luc, and p176-Luc and transactivate expression of the reporter gene. The lack of Nrl-mediated transactivation of p61-Luc is consistent with Nrl acting through NRE, which is present in p84-Luc but not in p61-Luc. Since the expression of rhodopsin is highly restricted in a cell type- and developmental stage-specific manner, one would predict that negative regulatory elements play a role in maintaining this high degree of specificity. The finding of decreased activity and responsiveness of p176-Luc and virtually no transactivation of p225-Luc does not prove but is consistent with this hypothesis. We speculate that repression of rhodopsin promoter function in CV-1 cells may be mediated by a negative regulatory factor that binds to sequences in the −225 promoter. Involvement of additional trans-acting factors (both positive and negative) in rhodopsin regulation *in vivo* is expected since Nrl expression in Y79 retinoblastoma cells is not sufficient for rhodopsin gene expression (ref. 11; D.J.Z., unpublished data). The cotransfection results also suggest that the transactivation can be mediated by the Nrl homodimer, although heterodimerization of Nrl with other proteins in CV-1 cells can not be ruled out. We believe that appropriate Nrl heterodimers in the retina may have a substantially higher transactivation effect on rhodopsin gene expression *in vivo*.

The specificity of Nrl-mediated transactivation was confirmed by cotransfection experiments using pMT-DD10 and pMT-NRLΔZ, constructs that expressed variant Nrl proteins. As predicted by the sequence, the truncated Nrl protein (t-Nrl) produced by pMT-DD10 inhibited the Nrl-mediated transactivation. Apparently, the presumptive Nrl–t-Nrl heterodimers and t-Nrl homodimers produced in the cotransfected cells do not have the ability to positively regulate promoter function, and inhibit transactivation, probably by competing with the Nrl homodimers for binding to the NRE. The presence of an alternatively spliced truncated form of the *NRL* mRNA (corresponding to the DD10 cDNA) in the human retina has been confirmed by reverse transcription–PCR and sequence analysis (A.S., unpublished data). Interestingly, other Maf proteins (e.g., MafK and MafF) also exist in forms that lack the putative N-terminal transactivation domain (17), and inhibitory truncated variants of several transcription factors have been reported (40–42). The naturally occurring t-Nrl protein may, therefore, have a physiological role as a negative regulator of the wild-type transcriptional activation function of Nrl.

The identification of Nrl as part of the retinal protein complex bound to the rhodopsin NRE and its ability to specifically transactivate the rhodopsin promoter in a heterologous cell culture system strongly argue for the involvement of Nrl in rhodopsin gene expression *in vivo*. Complementary studies in primary chick retinal cultures indicate that deletion or mutation of the rhodopsin NRE also reduces promoter activity and eliminates Nrl-mediated transactivation (R.K., D. Scheurer, E. Duh, A.R., A.S., R. Adler, and D.J.Z., unpublished data). Additionally, the expression pattern of *Nrl* during mouse retinal development is consistent with its predicted *in vivo* function in rhodopsin regulation. In differentiating retinal cells, the appearance of *Nrl* transcripts in the outer nuclear layer coincides with the formation of post-mitotic rod photoreceptors and precedes expression of the rhodopsin gene (46). Direct demonstration of a role for Nrl in rhodopsin gene regulation *in vivo*, however, will require transgenic approaches (31).

Since bZIP proteins are often involved in controlling a variety of genes and since *NRL* is expressed in other retinal cells, it seems likely that the Nrl protein plays a role in regulating genes in addition to rhodopsin. The high level of *NRL* expression observed in the adult retina supports this hypothesis. Further, the finding that changes in the structure and regulation of rhodopsin can cause retinal degeneration (43) suggests that mutations affecting Nrl function, which may alter the expression of rhodopsin or other important retinal genes, may also lead to retinal disease(s). In Sardinian autosomal recessive retinitis pigmentosa families, the disease locus has recently been linked to chromosome 14q11 markers (44), in close proximity to *NRL* (ref. 45; S. P. Dahl, W. J. Kimberling, and A.S., unpublished data).

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1. Barnstable, C. J. (1987) *Mol. Neurobiol.* **1,** 9–46.
2. Reh, T. A. (1992) in *Determinants of Neuronal Identity*, eds. Shankland, M. & Macagno, E. R. (Academic, San Diego), pp. 433–467.
3. Cepko, C. L. (1993) *Prog. Retinal Res.* **12,** 1–12.
4. Adler, R. (1993) *Invest. Ophthalmol. Visual Sci.* **34,** 1677–1682.
5. He, X. & Rosenfeld, M. G. (1991) *Neuron* **7,** 183–196.
6. Xiang, M., Zhou, L., Peng, Y.-W., Eddy, R. L., Shows, T. B. & Nathans, J. (1993) *Neuron* **11,** 689–701.
7. Hodgkinson, C. A., Moore, K. J., Nakayama, A., Steingrimsson, E., Copeland, N. G., Jenkins, N. A. & Arnheiter, H. (1993) *Cell* **74,** 395–404.
8. Beebe, D. C. (1994) *Invest. Ophthalmol. Visual Sci.* **35,** 2897–2900.
9. Liu, I. S. C., Chen, J.-d., Ploder, L., Vidgen, D., van der Kooy, D., Kalnins, V. I. & McInnes, R. R. (1994) *Neuron* **13,** 377–393.
10. Wang, S.-Z. & Adler, R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1351–1355.
11. Swaroop, A., Xu, J., Pawar, H., Jackson, A., Skolnick, C. & Agarwal, N. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 266–270.
12. Swaroop, A. (1993) in *Methods in Neurosciences: Photoreceptor Cells*, ed. Hargrave, P. A. (Academic, Orlando, FL), Vol. 15, pp. 285–300.
13. Farjo, Q., Jackson, A. U., Xu, J., Gryzenia, M., Skolnick, C., Agarwal, N. & Swaroop, A. (1993) *Genomics* **18,** 216–222.
14. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1988) *Science* **240,** 1759–1764.
15. Nishizawa, M., Kataoka, K., Goto, N., Fujiwara, K. T. & Kawai, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7711–7715.
16. Kurschner, C. & Morgan, J. I. (1995) *Mol. Cell. Biol.* **15,** 246–254.
17. Fujiwara, K. T., Kataoka, K. & Nishizawa, M. (1993) *Oncogene* **8,** 2371–2380.
18. Kataoka, K., Fujiwara, K. T., Noda, M. & Nishizawa, M. (1994) *Mol. Cell. Biol.* **14,** 7581–7591.
19. Andrews, N. C., Kotkow, K. J., Ney, P. A., Erdjument-Bromage, H., Tempst, P. & Orkin, S. H. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11488–11492.
20. Cordes, S. P. & Barsh, G. S. (1994) *Cell* **79,** 1025–1034.
21. Kataoka, K., Noda, M. & Nishizawa, M. (1994) *Mol. Cell. Biol.* **14,** 700–712.
22. Kerppola, T. K. & Curran, T. (1994) *Oncogene* **9,** 675–684.
23. Gorski, K., Carneiro, M. & Schibler, U. (1986) *Cell* **47,** 767–776.
24. Nie, Z., Chen, S., Kumar, R. & Zack, D. J. (1995) *J. Biol. Chem.*, in press.
25. Zack, D. J., Bennett, J., Wang, Y., Davenport, C., Klaunberg, B., Gearhart, J. & Nathans, J. (1991) *Neuron* **6,** 187–199.
26. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1991) *Current Protocols in Molecular Biology* (Wiley, New York).
27. Wise, R. J., Barr, P. J., Wong, P. A., Kiefer, M. C., Brake, A. J. & Kaufman, R. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9378–9382.
28. Kumar, R. & Zack, D. J. (1994) in *Molecular Genetics of Ocular Disease*, ed. Wiggs, J. (Wiley/Liss, New York), pp. 139–160.
29. Rehemtulla, A. & Kaufman, R. J. (1992) *Blood* **79,** 2349–2355.
30. Lem, J., Applebury, M. L., Falk, J. D., Flannery, J. G. & Simon, M. I. (1991) *Neuron* **6,** 201–210.
31. Zack, D. J. (1993) in *Methods in Neurosciences: Photoreceptor Cells*, ed. Hargrave, P. A. (Academic, Orlando, FL), Vol. 15, pp. 331–341.
32. Morabito, M. A., Yu, X. & Barnstable, C. J. (1991) *J. Biol. Chem.* **266,** 9667–9672.
33. Yu, X., Chung, M., Morabito, M. A. & Barnstable, C. J. (1993) *Biochem. Biophys. Res. Commun.* **191,** 76–82.
34. Sheshberadaran, H. & Takahashi, J. S. (1994) *Mol. Cell. Neurosci.* **5,** 309–318.
35. Nathans, J. & Hogness, D. S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4851–4855.
36. Kerppola, T. K. & Curran, T. (1994) *Oncogene* **9,** 3149–3158.
37. Igarashi, K., Kataoka, K., Itoh, K., Hayashi, N., Nishizawa, M. & Yamamoto, M. (1994) *Nature (London)* **367,** 568–572.
38. Engel, J. D. (1994) *Nature (London)* **367,** 516–517.
39. Struhl, K. (1991) *Neuron* **7,** 177–181.
40. Nakabeppu, Y. & Nathans, D. (1991) *Cell* **64,** 751–759.
41. Yen, J., Wisdom, R. M., Tratner, I. & Verma, I. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5077–5081.
42. Buettner, R., Kannan, P., Imhof, A., Bauer, R., Yim, S. O., Glockshuber, R., VanDyke, M. W. & Tainsky, M. A. (1993) *Mol. Cell. Biol.* **13,** 4173–4185.
43. Nathans, J. (1994) *Cell* **78,** 357–360.
44. Bruford, E. A., Mansfield, D. C., Teague, P. W., Barber, A., Fossarello, M. & Wright, A. F. (1994) *Am. J. Hum. Genet.* **55,** 181 (abstr.).
45. Yang-Feng, T. L. & Swaroop, A. (1992) *Genomics* **14,** 491–492.
46. Liu, Q., Ji, X., Breitman, M. L., Hitchcock, P. F. & Swaroop, A. (1996) *Oncogene* **12,** in press.