Characterization of the Xenopus Rhodopsin Gene*

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The abundant Xenopus rhodopsin gene and cDNA have been cloned and characterized. The gene is composed of five exons spanning 3.5 kilobase pairs of genomic DNA and codes for a protein 82% identical to the bovine rhodopsin. The CDNA was expressed in COS1 cells and regenerated with 11-cis-retinal, forming a light-sensitive pigment with maximal absorbance at 500 nm. Both Southern blots and polymerase chain reaction amplification of intron 1 revealed multiple products, indicating more than one allele for the rhodopsin gene. Comparisons with other vertebrate rhodopsin 5' upstream sequences showed significant nucleotide homologies in the 200 nucleotides proximal to the transcription initiation site. This homology included the TATA box region, Ret 1/PCE1 core sequence (CCAATTA), and surrounding nucleotides. To functionally characterize the rhodopsin promoter, transient embryo transfections were used to assay transcriptional control elements in the 5' upstream region using a luciferase reporter. DNA sequences encompassing -5500 to +41 were able to direct luciferase expression in embryo heads. Reporter gene expression was also observed in embryos microinjected with reporter plasmids during early blastomere stages. These results locate transcriptional control elements upstream of the Xenopus rhodopsin gene and show the feasibility of embryo transfections for promoter analysis of rod-specific genes.

In vertebrate retinas, the photoreceptor layer is made up of two morphologically and functionally distinct cell types, rods and cones (Dowling, 1987). In most vertebrates, there is a single class of rods and two to four different classes of cones. However, in amphibians there are two classes of rods: an abundant (principal) rod with spectral characteristics similar to rods from other species and a minor rod with blue-shifted absorption properties (Witkovsky et al., 1981). In all these cells, phototransduction is mediated by a group of proteins that control cGMP metabolism, e.g. opsin, transducin α subunit, arrestin, cGMP phosphodiesterase subunits, and cGMP channel (Hargrave and McDowell, 1992). There are isoforms of each of these proteins expressed in rods and others expressed in cones (Dowling, 1987; Lerea et al., 1986; Nir and Ransom, 1992; Hurwitz et al., 1985; Bognik et al., 1993). Studies on the developmental regulation of several of these genes demonstrate that the major control of expression occurs at the level of transcription initiation (Treichman et al., 1988; Timmers et al., 1993). However, the mechanism(s) regulating the photoreceptor cell-specific transcription in the vertebrate retina remain as yet unknown.

A number of studies have focused on the identification of cis-acting DNA elements in the promoters of photoreceptor genes using transgenic mice. Retina-specific reporter gene expression has been found using the 5' upstream regions of the bovine and mouse rhodopsin (Zack et al., 1991; Lem et al., 1991), human red and green opsin (Wang et al., 1992), human and mouse blue opsin (Chen et al., 1994; Chiu and Nathans, 1994a, 1994b) human IRBP (Liou et al., 1991), and mouse rod arrestin (Kikuchi et al., 1993) genes. Biochemical studies have identified and partially characterized nuclear proteins that bind to the 5' upstream regions of rhodopsin (Morabito et al., 1991; Yu et al., 1993; Sheshbadaran and Takahashi, 1994), transducin (Ahmad et al., 1994), and arrestin (Kikuchi et al., 1993). Three sites, Ret 1/PCE1, Ret 2/3, and glass-like, have been found in both mammalian and chicken rhodopsins. Additional transcription factors expressed in the retina have been identified by molecular cloning (Swaroop et al., 1992; Akazawa et al., 1992), and some bind to sequences in the rhodopsin gene (reviewed in Kumar and Zack, 1995). These studies suggest that control of photoreceptor gene transcription may involve a number of different transcription factors that act in concert to produce the unique developmental and cell-specific expression pattern.

Xenopus offers a number of specific advantages as a model system in which to study molecular mechanisms that regulate retinal development and gene expression. First, Xenopus embryology and development has been described in great detail (Nieuwkoop and Faber, 1967). Embryos can be produced in vitro and develop completely outside the female, thus allowing precise manipulation at any time after fertilization (Hamburger, 1960). Second, retinal development proceeds quickly; precursor cells develop to produce the layers of the adult retina by about 3 days post-fertilization (Holt et al., 1988). Third, foreign genes can be introduced into the retina and brain of Xenopus embryos by microinjection techniques or transfection with Lipofectin (Holt et al., 1990; Harris et al., 1995). To study the mechanisms of rod-specific transcription of phototransduction genes, we report here the cloning and characterization of the Xenopus rhodopsin gene, encoding the most abundant phototransduction protein. Furthermore, we show that sequences upstream of the Xenopus rhodopsin gene drive the head-specific expression of a reporter gene in transient transfections of developing Xenopus embryos using Lipofectin (Holt et al., 1990) and by microinjecting early stage blastomeres (Huang and Moody, 1993). This approach will allow a comprehensive study of the cis-acting elements in Xenopus phototransduction genes.
EXPERIMENTAL PROCEDURES

cDNA Clones—An oligo(dT)-primed cDNA library, in λZapI (Stratagene), was made from adult Xenopus (Nasco, Ft. Atkins, WI) female retinal RNA prepared by acid-guanidinium extraction (Chomczynski and Sacchi, 1987). A 1.6-kb bovine opsin fragment (Nathans et al., 1983) was radiolabeled according to the random primer method using the Premix kit (Stratagene) and (α-32P)dATP (3000 Ci/mmol, DuPont NEN). Hybridization was performed in 50% deionized formaldehyde, 1 M NaCl, 10 mM NaHCO3, pH 7.4 (0.1× SSC, 2.5 mM EDTA), 10% dextran sulfate, 5 × Denhardt’s solution, 0.5% SDS at 42°C for 16 h. The filters were washed at a final stringency of 1 × SSPE and 0.5% SDS at 42°C. Positive plaques were isolated and purified, and inserts were obtained by in vivo rescue. The longest clone, pXOP71, was analyzed by restriction mapping and sequencing using dideoxy chain termination method and Sequenase (U. S. Biochemical Corp.). Using antisense primers: P2, 5′-catgctagacagggacga; and P6, 5′-atgtagacaggctgatg, RACE PCR (Frohman et al., 1988) was carried out on retinal poly(A)+ RNA. PCR DNA fragments were excised from 1% low melting point agarose gels and cloned as described (Marchuk et al., 1991). One PCR clone was used for sequencing and also to screen the cDNA library, from which one positive clone was also characterized. Sequences were determined on both strands.

Expression of Xenopus Opsin cDNA in COS1 Cells—A Xenopus opsin cDNA expression construct was assembled in a mammalian expression vector as follows. First, a complete cDNA was constructed by ligating a BamHI-BstEII RACE PCR fragment into pXOP71. Then, an Apo-HindIII linker fragment containing nucleotides 111-1153 (encoding amino acids 5-347) of the Xenopus opsin cDNA was ligated into the EcoRI-Sall of pmTS (Khorana et al., 1988) using synthetic nucleotide adapters. The final product, pM-TXOP, consists of nucleotides 1-11 from the synthetic bovine rhodopsin gene (encoding amino acids 1-4, which are identical in bovine and Xenopus; see Ferretti et al., 1988), nucleotides 111-1153 (encoding amino acids 5-347) of the Xenopus opsin and nucleotides 1000-1047 (encoding amino acids 334-348 and the stop codon) from the synthetic bovine rhodopsin gene. This fusion protein has 361 amino acids and contains the binding site for the monomodal antibody ID4 (Molday and Mackenzie, 1983). The construct was confirmed by DNA sequencing. Transient transfections of COS1 cells and rhodopsin purification were performed as described (Karnik et al., 1993). UV-visible spectra were recorded using a Beckman DU 600 single-beam spectrophotometer and displayed using SigmaPlot software (Jandel). 

Genomic Clones—A Xenopus genomic library in λDASH, (gift of E. DeRobertis, UCLA) was screened with the radiolabeled RACE PCR product. Positive plaques were isolated, purified, and restriction-mapped according to standard protocols (Sambrook et al., 1989). The opsin gene was used as the probe. Numerous partial clones were found on two fragments, 5.8 and 5.5 kb, which were subcloned into pBluescriptII (Stratagene). The rhodopsin gene and 1.2 kb of upstream region were sequenced on both strands. Sequence analysis was performed using University of Wisconsin NCC software package.

RNA Analysis—Primer extension reactions were carried out using the protocol described by the manufacturer (Life Technologies, Inc.), Extenson of Xenopus adult total retinal and brain RNA was carried out using two antisense primers: P9, 5′-gatcctgactggctcctgc; and P10, 5′-gtctggctagtgatgac, in the first exon of the rhodopsin gene. 5 μg of adult Xenopus total retinal and brain RNA were extended with 2 pmol each of end-labeled P9 and P10 in separate reactions (~220-fold excess of primer). The annealing and extension reaction was performed at 42°C for 30 min. To control for specificity of primer binding, retinal RNA was also incubated with 50-fold excess of the corresponding unlabeled primer. Additional experiments using 25-200 ng of adult Xenopus retinal poly(A)+ RNA were carried out. The products were resolved on 8% acrylamide gels.

Genomic Analysis—Genomic DNA was prepared from Xenopus testes (Sambrook et al., 1989) and digested with restriction enzymes overnight, and 16 μg was run on a 0.7% agarose gel. After transfer to nylon, the blot was hybridized with 32P-radiolabeled RACE product. Final washes were performed at 62°C in 0.1× SSC containing 1% SDS. The blot was exposed using a PhosphorImager (Molecular Dynamics). Polyacrylamide chain reaction was carried out using exon-specific primer pairs: P9, 5′-caagccacatgatgagctgtatcg; and P12, 5′-caagccacatgatgagctgtatcg in exon 2 to amplify the first intron. Amplifications were carried out using 300 ng of adult Xenopus genomic DNA or 150 ng of the nation basal level.

The abbreviations used are: kb, kilobase pair(s); RACE, rapid amplification of cDNA ends; PCR, polymerase chain reaction; bp, base pair(s); RLU, relative light units.

Xenopus Rhodopsin Gene

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identity and 92% similarity to bovine rhodopsin (Fig. 2). The sequence of XOP differs from a Xenopus rhodopsin isolated from another cDNA library (Saha and Grainger, 1992) at a number of locations: 6 nucleotides in the coding region (resulting in three variant amino acids: Gin-107 → Pro, Met-137 → Ile, and Ala-241 → Leu); 12 positions, including several deletions, in the 3'-untranslated region; and 3 positions in the 5'-untranslated region, including a 222-nucleotide 5'-extension. Some of these variants were observed in the studies reported here, either in cDNA from adult retinal tissue or genomic clones (see below), which matched XOP1.

In order to demonstrate that XOP1 is the rhodopsin found in the abundant (principal) rod cell, the cDNA was expressed in COS1 cells and the UV-visible absorbance properties determined. To facilitate purification of the expressed protein, the C-terminal 7 amino acids were replaced with the last 14 amino acids of bovine rhodopsin, introducing the epitope for the monoclonal antibody ID4 (Molday and Mackenzie, 1983) into XOP1. After transient transfection of COS1 cells and incubation of the cells with 11-retinal, the visual pigment was solubilized with dodecyl maltoside and purified by immunoaffinity chromatography (Oprion et al., 1987). The UV-visible absorbance spectra had a maximal absorbance at 500 nm, identical to bovine rhodopsin (Fig. 3). This value agrees with that obtained from microspectrophotometry of the abundant rod in Xenopus retina following regeneration with 11-cis-retinal (Witkovsky et al. 1981). Thus, XOP1 encodes the abundant rhodopsin in the adult Xenopus retina.

Characterization of the Rhodopsin Gene—To obtain the rhodopsin gene and upstream sequences, a Xenopus genomic library was screened with a 178-bp BamHI-NcoI fragment containing the 5' end of the cDNA. One of the genomic clones obtained from this screen had a 19-kb genomic insert (Fig. 1). Analysis of the insert indicated that there were approximately 13 kb upstream of exon 1, which was mapped to the 5.8-kb BamHI fragment. Two BamHI fragments, containing the cDNA sequence and 5' untranscribed regions, were subcloned and partially sequenced. The Xenopus rhodopsin gene is 3507 bp and has five exons, with the four introns having sizes of 248, 601, 250, and 705 bp (Figs. 1 and 2). The positions of the intron-exon junctions in the gene were determined (Table I) and are conserved with other known rhodopsin genes. The sequence of the exons in the gene was identical to that obtained from the cDNA, indicating that the opsin genomic clone encoded the abundantly expressed rhodopsin in the adult retina, XOP1.

The size of the mRNA was determined by Northern analysis, which showed a single band of 1.7 kb found only in retinal RNA (Fig. 4A). This size is similar to that found to be expressed in tadpole heads (Saha and Grainger, 1992). In order to determine the transcription initiation site, primer extension was performed with two different antisense primers, P9 and P10. A number of extension products differing in their relative intensities were obtained using retinal RNA (Fig. 4B). The major extension products were 42 bp with P9 and 120 bp with P10, and this nucleotide is designated as +1 (Fig. 2). Although the size of the extension product agrees with that found by RACE PCR, the gene contains a T instead of a G as found in the cDNA at +1. The transcription initiation site was also confirmed using poly(A)^+ RNA and P9; the largest extension product mapped to +1, although the major product mapped to +2. The differing intensities found in the two RNA preparations may reflect heterogeneity in the frogs used to prepare the different samples, or slight differences in primer specificity in the two preparations. There were additional minor products, reproducibly found in primer extensions, that occurred at +5 and +6 (Fig. 3B). The existence of multiple extension products has been reported for a number of genes and is consistent with the lack of a consensus TATA box in the rhodopsin gene (see below).

Rhodopsin genes characterized thus far are present in a single copy in the genome. However, Xenopus contains a pseudotetraploid genome (Graf and Kobel, 1991), which raises the possibility of multiple copies for rhodopsin in this species, all of which might be expressed in the retina. To investigate this possibility, Southern blots were performed using Xenopus genomic DNA. Using three different enzymes, multiple bands of similar intensity, including the band expected from genomic clone gopR1, were observed, even after high stringency washing (Fig. 5A). This suggests that there are four alleles of rhodopsin in Xenopus. Further evidence for multiple alleles was found when intron 1 of the rhodopsin gene was amplified from Xenopus genomic DNA by PCR. Four products, of sizes 368, 500, 550, and 650 bp were found (Fig. 5B, lane 1). Comparisons with the control XOP1 phase (lane 6) identified the 368-bp product as arising from this gene. The 500- and 550-bp products amplified to the same level as the 368-bp product, while the 650-bp product was slightly less intense. Comparison of the primer sequences with the Xenopus violet cone opsin2 and with other cone opsins from chicken (Okano et al., 1992) showed little homology and thus would not be expected to amplify under these PCR conditions. Thus, in Xenopus, there are at least four genes encoding rhodopsin or a highly homologous opsin perhaps expressed in the green rod (Witkovsky et al., 1981) further work is under way to obtain the sequence of the novel PCR products.

Upstream Sequence Analysis—The sequence upstream of the transcription initiation site was analyzed for general transcriptional control elements and for homology with other vertebrate rhodopsin genes. The Xenopus upstream region does not contain a TATA box (Wingender, 1990), instead a TTTAAA sequence surrounded by G-rich sequences is present at −31 position. In addition, the upstream sequence had no homology to any of the described transcriptional initiators (Weiss and Reinberg, 1992). Consensus sites for several general trans-activating factors were found (Fig. 2), notably two sites for AP1 (TGANT/A/C, Jones et al., 1988) at −99 and −292, SP1-like GC boxes (Briggs et al., 1986; Jones et al. and Tijan, 1985) at −390 and −365 and a single CREB site (ACGTCA; Sassone-Corsi (1988)) further upstream at −907. Significant homology with

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five other rhodopsin sequences: bovine, human, and mouse (Zack et al., 1991), rat (Yu et al., 1993), and chicken (Sheshberadaran and Takahashi, 1994), was found from 210 to 2180 (Fig. 6). Overall sequence identities ranged from 56% (chicken) to 39% (rat) for this region, with much higher values in localized segments (Fig. 6, shaded regions; e.g., 75% in the 15 nucleotides surrounding the TATA consensus). The Ret 1/PCE1 core sequence (CCAATTA), present in many genes expressed in the retina (Kikuchi et al., 1994), was found at 2133. Moreover, the flanking sequences (2164 to 2122) also show strong conservation across the rhodopsins, and immediately downstream there is a highly conserved AP1 site at 2199. A strong match to a glass-like sequence, shown to bind neural nuclear proteins in both chicken (Sheshberadaran and Takahashi, 1994) and Drosophila (Moses and Rubin, 1991) rhodopsin genes is present at 2194 (Fig. 6B). A second weaker homology was found to a second glass-like element further upstream (Fig. 6B). Three additional sites are apparent in the Xenopus upstream regions (Fig. 6C): one homologous to the rat Ret 2 protected sequence (Yu et al., 1992) at 2330, and two to a human retina-specific leucine zipper (NRL) transcription factor (Swaroop et al., 1992). In mammalian rhodopsins, a highly conserved 85-bp sequence...
is found more than 1 kb upstream (Zack et al., 1991). No significant homology was apparent in the Xenopus sequence. However, it is possible that it may be contained further upstream or that sequence divergence has made identification difficult. Comparisons to the distal region of the chicken rhodopsin (Sheshberadaran and Takahashi, 1994), including two sequences previously described to have weak homology to mammalian sequences, showed no significant matches. In summary, a number of potential regulatory sequences, with nuclear protein binding activity, functional activity, or homology with known general transcriptional control elements, are found within the proximal 400 bp in the Xenopus rhodopsin, strongly suggesting a role for this region in the cell-specific expression in the retina. These results also highlight the striking similarity found in rhodopsin promoter regions in many vertebrates and reveal a potentially conserved regulatory mechanism.

**Xenopus Rhodopsin Upstream Sequence Directs Luciferase Expression**—In order to identify functional genomic sequences in the Xenopus rhodopsin gene that control its transcription, a transient embryo transfection assay was used. A genomic BamHI fragment (Fig. 1), containing 5.5 kb of 5' upstream sequences including 41 nucleotides of the 5'-untranslated region, was cloned in both orientations into a luciferase reporter plasmid, pGL2 and used in a transient embryo transfection assay. DNA was introduced into Xenopus embryos, at stages (26/27) when 80–90% of the retinal precursor cells are still dividing (Holt et al., 1988). Two preparations of embryo heads were used in the transfection procedure (Holt et al., 1990):

**EDTA-treated whole heads and manually peeled heads. In order to increase access to the eye vesicle, both preparations of embryo heads were trypsinized for 90 s prior to the addition of a mixture of DNA and Lipofectin (Holt et al., 1990). Heads were cultured to stage 42 when functional photoreceptors are present in the Xenopus retina (Witkovsky et al., 1976). In experiments using EDTA-treated heads, pXOP(−5500/+41)luc showed luciferase activity > 35-fold higher than from the promoterless control, pGL2 (Fig. 7, Experiment A), which did not differ from that observed in untransfected heads (data not shown). Further, in transfections of EDTA-treated heads, luciferase activity from pXOP(−5500/+41)luc was 4–15% of that observed with the general promoter, cytomegalovirus. The activities observed with pXOP(−5500/+41)luc in two independent transfections were comparable, suggesting that EDTA treatment of the heads permitted consistent access to retinal precursors. To test whether pXOP(−5500/+41)luc could express luciferase in non-retinal cells, stage 26/27 trunks treated with EDTA were transfected and assayed as before. In contrast to the heads, pXOP(−5500/+41)luc did not express luciferase activity in the trunk above that produced by the promoterless plasmid, pGL2, even though a high level of luciferase activity was observed with pCMVluc (Fig. 7, Experiment C).
FIG. 6. Homologies with other vertebrate rhodopsin upstream sequences. A, proximal sequence homology. Sequence alignment of the immediate upstream nucleotides of the Xenopus (XEN), chicken (CHK), human (HUM), bovine (BOV), rat (RAT), and mouse (MUS) rhodopsin genes shown. Alignments were created using a window size of 6 and a stringency of 67%. Gaps introduced in the sequence for optimal alignment are shown by dots. Regions containing greater than 75% identity across species are shaded. Transcription start sites (boxed nucleotides) and position of the initiator methionine (arrow) are indicated. Potential GC boxes binding SP1 are indicated with dotted underline and pyrimidine tracts with solid underline.

B Glass element

Proximal

Chicken -744 TTTTACATCAGCTAACCCTTCAAATATGAGGCTGTCGACGG

Xenopus -301 GGGAAATTCGGAAACATCATCAGCACTGGCTGACGG

Drosophila -223 CCCACTGGAGGCACTCCCTGAAATGAGGCTGTCGACGG

Distal

Chicken -1118 TGGGAACATCTCAGTTTGCAGGTCACTGGTGACGG

Xenopus -920 AACACGACATCAGAGGTGGCTACGCAGGACGG

Drosophila -188 CATATGTTAAAGGCTACATCAGTTTGCAGGTCACTGGTGACGG

C Ret 2 and NRL

Rat Ret 2 -1461 CCTTAACTCAGCTAACCCTTCAAATATGAGGCTGTCGACGG

Xenopus -188 TGTTTACAGGCACTCCCTCAG

Human NRL -67 TGCTGATTCAGCGCAGCGGAGCAGCTGAGGG

Xenopus -412 TGCTGATTCAGCGCAGCGGAGCAGCTGAGGG

Fig. 6. Homologies with other vertebrate rhodopsin upstream sequences. A, proximal sequence homology. Sequence alignment of the ~450 immediate upstream nucleotides of the Xenopus (XEN), chicken (CHK), human (HUM), bovine (BOV), rat (RAT), and mouse (MUS) rhodopsin genes is shown. Alignments were created using a window size of 6 and a stringency of 67%. Gaps introduced in the sequence for optimal alignment are shown by dots. Regions containing greater than 75% identity across species are shaded. Transcription start sites (boxed nucleotides) and position of the initiator methionine (arrow) are indicated. Potential GC boxes binding SP1 are indicated with dotted underline and pyrimidine tracts with solid underline. B, nucleotide identities of the Xenopus upstream sequence with glass elements, proximal and distal, with core sequences underlined. Nucleotides conserved between Xenopus and chicken are shown in italics, and across all three species are indicated in bold. C, homologies of the Xenopus rhodopsin upstream sequence with the human retinal leucine zipper binding sequence, NRL, and rat Ret2 are shown with nucleotide identities in bold.
Trypsinization and lipofection were dissected and the head epidermis was manually removed prior to embryos were dissected and treated with trypsin in the presence of levels obtained from transient expression experiments. Stage 26/27 XOP1 and GL2 is the (promoterless) control plasmid.

The transcriptional activity of the 5.5-kb rhodopsin upstream sequences direct transient expression of luciferase in Xenopus embryos. A, the luciferase reporter constructs are diagrammed with the luciferase gene (luc) transcribed from left to right. Solid boxes indicate genomic sequences from XOP1 and GL2 is the (promoterless) control plasmid. B, Luciferase levels obtained from transient expression experiments. Stage 26/27 embryos were dissected and treated with trypsin in the presence of EDTA prior to lipofection (Experiments A and C). Additional embryos were dissected and the head epidermis was manually removed prior to trypsinization and lipofection (Experiment B). Embryonic tissue was incubated to the equivalent of stage 42/43 and assayed for luciferase activity. Activities are presented as RLU/embryo, where 1 pg of luciferase = 85,000 RLU. Early stage blastomeres (8-cell or 32-cell, Experiment D or E, respectively) were injected with plasmid and cultured to stage 42, when luciferase levels were determined.

to EDTA-treated heads, the high level of luciferase activity observed with pCMVluc was due to the 4-fold greater amount of protein used in the assays. In order to test whether the opposite orientation of the ops1 upstream sequence could drive the expression of luciferase, EDTA-treated heads were transfected with pXOP(+41/-5500)luc and only background luciferase activity was observed (Fig. 7). Thus, the ability of the 5.5-kb rhodopsin upstream sequence to drive the head-specific expression of luciferase in an orientation-dependent manner indicates the presence of transcriptional control elements within this region.

The transcriptional activity of the 5.5-kb rhodopsin upstream fragment was also tested in another preparation of embryo heads whose outer epidermal layer had been manually peeled to potentially improve the transfection efficiency in the eye vesicle. This preparation gave an average of a 3-fold enhancement in the luciferase activity driven by pCMVluc compared to that observed in EDTA-treated heads. However, luciferase activity from pXOP(-5500/+41)luc varied widely (Fig. 7, Experiment B). Therefore, although improved access to retinal precursors is achieved by peeled heads, uncontrolled variation makes the study of the ops promoter difficult.

To specifically target pXOP(-5500/+41)luc to a large population of retinal precursor cells, the reporter DNA was micro-injected bilaterally into cleavage stage blastomeres that contribute significant numbers of cells to the stage 42 retina (D1, 8-cell embryo; D11, 32-cell embryo; Kline and Moody (1990) and Huang and Moody (1993)). Following injection of the plasmid DNA, embryos were cultured to stage 42 and assayed for luciferase activity as before. In injected 32-cell embryos, luciferase activity observed using pXOP(-5500/+41)luc was >200-fold higher than that obtained using pGL2 (Fig. 7, Experiments D and E). Comparable luciferase activity was also observed in embryos injected at the 8-cell stage with pXOP(-5500/+41)luc and this was 2.8% of that obtained using pCMVluc. Therefore, luciferase activity observed upon blastomere injection of retina progenitor blastomeres with pXOP(-5500/+41)luc confirms the transcriptional activity of rhodopsin upstream sequence observed in transient embryo transfections. Further, blastomere injections yielded >3-fold higher luciferase activity using pXOP(-5500/+41)luc than that observed in transient transfection of EDTA-treated heads.

Taken together, the results of these three approaches: transfection of EDTA-treated heads, transfection of peeled heads, and blastomere injection, indicate that the 5.5-kb fragment contains transcriptional control sequences of the rhodopsin gene.

**DISCUSSION**

As a first step toward identifying cis-acting elements controlling the rod cell-specific expression of the Xenopus rhodopsin gene, we have characterized the gene and expression products, and identified transcriptional activity in upstream sequences. The Xenopus gene XOP1 has an overall structural organization conserved with other vertebrate rhodopsin genes. Both sequence comparisons and functional expression of the cDNA in COS1 cells has identified XOP1 as encoding a rhodopsin. Xenopus has two rod cells expressing distinct rhodopsins (Rohlich et al., 1989), one absorbing at 520 nm (red rods) and other at 445 nm (green rods, Witkovsky et al., 1981). The red rod is by far the more abundant cell, outnumbering green rods by greater than 10-fold (Rohlich et al., 1989). The abundance of XOP1 in the retinal cDNA library suggested that it is the rhodopsin in the abundant rod cell. By expressing the cDNA in COS1 cells, we have identified the absorbance maximum of XOP1 to be 500 nm, when regenerated with 11-cis-retinal (A). This is the wavelength of the abundant red rod pigment, when measured in retinas that were bleached and then regenerated with 11-cis-retinal (Witkovsky et al., 1981). Normally, Xenopus visual pigments are formed from 11-cis-dehydroretinal (A), which leads to a 20-nm red shift in the absorption maximum (Witkovsky et al., 1981). Thus the COS1 transfection experiments show that XOP1 encodes the rhodopsin from the red rod.

A Xenopus rhodopsin cDNA (Saha and Grainger, 1992) has been isolated from a tadpole library and has several nucleotide and amino acid differences with XOP1. Although Southern blots and PCR of genomic DNA suggests multiple alleles for rhodopsin (Fig. 5), efforts to identify the unique sequences by PCR using a primer to the tadpole 5’-untranslated region were unsuccessful (data not shown). Based on the unusually high degree of sequence identity, both in the coding and untranslated regions, it is unlikely that the tadpole cDNA and the one reported here arise from different genes since most Xenopus alleles show more than 4% variation in the coding region (Graf and Kobel, 1991). Moreover, the 5’ nucleotides (1-222 nucleotides) found in the tadpole cDNA are most similar to an untranslated gene (data not shown), and thus are probably an artifact of library construction. The source of other variants is unclear. Isolation of additional rhodopsin alleles, for example using PCR of intron 1, will permit complete characterization of rhodopsin genes in Xenopus.
To study the Xenopus rhodopsin promoter, we have developed and utilized an assay based on transient embryo transfections, allowing the analysis of retina-specific gene expression in intact Xenopus embryonic tissue. Using this approach, we have seen high levels of reporter gene expression in heads transfected with 5.5 kb of upstream sequence. We have further shown that as little as 600 bp also efficiently drives luciferase expression in this assay. Moreover, we have extended this approach to other Xenopus genes, including transducin α-subunit. Studies of mammalian retinal genes have commonly employed transgenic mice, which require a number of individual lines and also exhibit position effects of the introduced transgene. Additionally, retinal cell lines and dissociated primary cell systems for rhodopsin promoter studies are done outside the normal conditions for retinal development. Thus, the transient embryo transfection-based promoter assay provides an alternate method for the detection of transcriptional activity from genomic sequences. When combined with the use of blastomere injection, it provides a quick and powerful approach to other mammalian promoters.

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