Xenopus Rhodopsin Promoter

IDENTIFICATION OF IMMEDIATE UPSTREAM SEQUENCES NECESSARY FOR HIGH LEVEL, ROD-SPECIFIC TRANSCRIPTION

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To understand the mechanisms that control the cell-specific visual pigment gene transcription, the Xenopus rhodopsin 5′ regulatory region has been characterized in vivo using transient transfection of Xenopus embryos and transgenesis. The principal control sequences were located within −233/+41, a region with significant conservation with mammalian rhodopsin genes. DNase footprinting indicated seven distinct regions that contain potential cis-acting elements. Sequences near the initiation site (−45/+41, basal region) were essential, but not sufficient, for rod-specific transcription. Two negative regulatory regions were found, one between −233 to −202, with no apparent similarity to known elements, and a second Ret-1-like CAAT (−136/−122) motif. Deletion of either sequence led to a 2–3-fold increase in expression levels, without a change in rod specificity. Sequences between −170 to −146, which contain an E-box motif, were necessary for high level expression in transgenic tadpoles but not in transient transfections. Sequences between −84 and −58, which contained an NRE-like consensus were found to be necessary for high level expression in both assays. Although expression levels were modulated by various proximal sequences in the rhodopsin promoter, none of the tested sequences were found to be necessary for rod specificity. Promoter constructs with a consensus BAT-1 sequence in conjunction with an NRE-like element upstream of the basal promoter directed low level green fluorescent protein expression in the central nervous system in transgenic tadpoles. These results suggest that rod cell-specific expression of rhodopsin is controlled by redundant elements in the proximal promoter.

Phototransduction occurs in the photoreceptor layer of the vertebrate retina, which is composed of distinct cell types: rods and cones (1). These cells express a number of specific proteins that regulate the light-dependent currents mediating vision (2, 3). Among these cell-specific proteins are the visual pigments, which combine with 11-cis-retinal to form the light-sensitive component of the transduction cascade. The visual pigments are a large family of genes, which contain rod-specific rhodopsins and at least four classes of cone-specific opsins (4). Rhodopsin, required for nocturnal vision, is the most abundant opsin in many vertebrate retinæ by virtue of the size of the rod outer segments, abundance of the rod cells, and the high level of transcription. As such, the regulation of rhodopsin expression has been a focus for understanding mechanisms of cell-specific gene expression in the retina (5).

Transcription initiation has been identified as the major control point for rhodopsin gene expression (6, 7). A variety of studies using different approaches have demonstrated that important transcriptional control sequences lie within the 5′ upstream regions of various rhodopsin genes. Functional assays using transgenic mice have shown that 2–4 kb1 of upstream sequences from the mouse and bovine rhodopsin genes directly regulate gene expression to the photoreceptor layer (8, 9), with sequences proximal to the initiation site (−500 and −222) being sufficient for retina-specific expression. The importance of the proximal sequences is highlighted by the high degree of homology found in this region among vertebrate rhodopsins (10). However, the immediate upstream sequences from the mammalian opsins were not able to limit expression of the reporter to rods as expected for rhodopsin (11, 12). Expression levels are also regulated by a sequence termed rhodopsin enhancer region, located −2 kb upstream of the initiation site (13). The binding of retina-specific nuclear factors to rhodopsin upstream sequences have been localized in both proximal and distal upstream regions, suggesting a role for these elements in regulating expression (5). However, a complete description of the cis-acting elements that control transcription in rod photoreceptors is not yet available. Transcription factors that potentially regulate gene expression have been identified in the mammalian retina and several have been shown to activate rhodopsin expression in heterologous systems, e.g. Nrl (14, 15), Crx (16), and Erx (17). The mechanisms by which the different

1 The abbreviations used are: kb, kilobase pair(s); bp, base pair(s); XOP, Xenopus rhodopsin gene; RLU, relative light unit(s); luc, luciferase; GFP, green fluorescent protein; Nrl, neural retinal leucine zipper transcription factor; Crx, Cone-rod homeobox transcription factor; Erx, Empty spiracles-related homebox; Rx, Pax6-related homebox; CMV, cytomegalovirus; PCR, polymerase chain reaction; EGFP, enhanced green fluorescent protein; CAR, cardiac actin.

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cis-acting elements in the rhodopsin upstream regions function, either independently or in concert, to produce rod-specific expression are not known.

We have used Xenopus embryos for transient transfection studies and transgenesis to investigate rod-specific transcription. Previously, we found that a 5.5-kb rhodopsin upstream fragment was transcriptionally active, driving the expression of reporter both in Xenopus embryo transfections (10) and in transgenic frogs (18). In this paper, we map the rhodopsin functional limits of the promoter to the proximal region spanning nucleotides to −85 by using mutational analysis and DNase footprinting to define the functional limits of the promoter to the proximal regions of the rhodopsin promoter−85 to −58. We report the cloning of synthetic oligonucleotides (IDT, Coralville, IA). pXOP(41)GFP was generated by subcloning a XOP promoter fragment was transcriptionally active, driving the expression of reporter both in Xenopus embryo transfections (10) and in transgenic frogs (18). In this paper, we map the functional limits of the promoter to the proximal −30 region spanning nucleotides to −58 by using mutational analysis and DNase footprinting to define the functional limits of the promoter to the proximal −30 region spanning nucleotides to −58 by using mutational analysis and DNase footprinting to define the functional limits of the promoter to the proximal regions of the rhodopsin promoter−85 to −58. No sense primer was necessary for this construct (see “Experimental Procedures”).

**EXPERIMENTAL PROCEDURES**

**DNA Constructs**

**Upstream Fragments**—The plasmids pXOP(5500/+41)lac and pXOP(+41/+5500)lac contain the 5.5-kb upstream sequences from the Xenopus rhodopsin gene, XOP, in the forward and reverse directions, respectively, in pGL2 vector (Promega, WI) and were constructed as described previously (10). pXOP(+1300/+41)lac was derived from pXOP(5500/+41)lac by SacI digestion and religation of the 6.9-kb vector-containing fragment. pXOP(−503/+41)lac was derived from pXOP(−1300/+41)lac by digestion with PstI and KpnI. The resulting ends were filled-in using Klenow DNA polymerase and religated. pXOP(−503/+41)lac (5800) was generated by cloning a 5.8-kb BamHI genomic fragment containing the Xenopus rhodopsin exons and downstream sequences, into the BamHI site downstream of the luciferase gene in pXOP(−503/+41)lac.

**Deletions**—The deletion series was constructed using exonuclease III and manganese nuclease digestion (Strategene). The plasmid containing a deletion of the TATA box region, pXOP(−503/−46)lac, was constructed by digestion of pXOP(−503/+41) with HindIII and subsequent religation.

**Basal Region Constructs**—The TATA box region was isolated as a HindIII fragment from pXOP(−503/+41)lac and cloned into pGL2 in both orientations, pXOP(−46/+41)lac and pXOP(+/−46)lac, as well as a dimer, pXOP(−46/+41)lac. All deletion constructs were sequenced using the dyeoxy chain termination method to confirm the sequence and orientation of inserts. For transgenic experiments, pXOP(−508/+41)GFP was generated by subcloning a PstI-BamHI fragment into pEGFP(−18).

**Targeted Disruptions**—Targeted disruptions of conserved regions in the Xenopus rhodopsin promoter (−503/+41) were generated using a PCR-based approach using primers (Table I) with a PstI overhang (19). To generate the Δ(−85/−58), the PCR primer (Table I) contained a HindIII overhang, permitting direct cloning of the product into pXOP(−502/+41)lac. A second construct that disrupted −84 to −58 but maintained the spacing of the native promoter was made by cloning a synthetic oligonucleotide (5’−CTGGTACGGAGGCTCTCAGCATCCAGCAATGGTCA−3’ into the PstI site in the Δ(−85/−58) construct. PCR was performed using Ultra DNA polymerase (PerkinElmer Life Sciences). The 5’ products were digested with PstI and PstI-BamHI, and constructs were generated by three-part ligation of PstI-PstI product with the PstI-BamHI product. These were cloned directionally into the PstI-BamHI sites of pGL2 (Promega, WI). All mutant promoter constructs were verified by sequencing. The Δ(−136/−122) contained a single base change (G to C at −396) in addition to the intended replacement of the Ret-I site by PstI.

**GFP Constructs**—The XOP basal region (−52/+41) was cloned as a HindIII-BamHI fragment into complementary sites in pEGFP (−18) to generate EGFP-XOP basal. This plasmid was digested with PstI and HindIII and gel-purified. Rhodopsin-targeted deletion luciferase constructs were digested with HindIII/HindIII and cloned into the pXOP(−52/HindIIIdut II) sites of pEGFP(XOP) to generate rhodopsin-targeted deletion constructs in EGFP. The Δ(−52/−38) HindIII fragment was cloned directly into EGFP digested with the same enzymes. The clones were verified by restriction digestion and sequencing. Plasmid preparations were performed using the Qiagen protocol (Chatsworth, CA).

**Embryo Transfections**

Embryos were obtained by in vitro fertilization using hormonally induced adult Xenopus females (Nasco), dejellied (21), and grown at 18–24°C in sterile media (50% DME, 25% Ringer, 25% TEA, pH 7.8, NaCl, 2 mM KCl, 1 mM MgSO4, 2 mM CaCl2, 5 mM HEPEs, 0.1 μM 2,3-dioleyloxypropyl-N,N,N-trimethylammonium methysulfate (Roche Molecular Biochemicals) were used during these studies and the amount of DNA and the DNA:lipid ratio were 6–12 μg.
and 1.3, respectively. DNA used for transfections were either purified by twice banding in CaCl (24) or by the Qiagen protocol. At least two different preparations were tested for each plasmid. Groupwise comparisons of mean activities (RLU/8ead) generated in individual transfection trials were performed using single-factor analysis of variance (a = 0.05 for planned comparisons; Ref. 25). Transfection of trunks and whole embryos were done as controls. For luciferase assay, the heads (6–10) were homogenized and aliquots were assayed (using a protocol supplied by Promega) to determine the picograms of luciferase produced per well. The luciferase assay gave 50,000–100,000 relative light units/μg of protein (Sigma)/30 s using the single-channel luminometer (Berthold). Protein estimations were done using the Bradford reagent (Bio-Rad) and bovine serum albumin standards, and ranged from 10 to 20 μg/μl. Luciferase activity is reported as the mean (including all trials, n given in figure legend) ± S.E.

Transgenic Xenopus

DNA was digested with XhoI to linearize the plasmid and purified after digestion (High Pure PCR Product Purification Kit, Roche Molecular Biochemicals), with final elution in water. Transgenic Xenopus embryos were produced using restriction enzyme-mediated integration (16, 27). 32P-End-labeled DNA fragments of the various preparations were tested for each plasmid. Groupwise comparisons of mean activities (RLU/8ead) generated in individual transfection trials were performed using single-factor analysis of variance (a = 0.05 for planned comparisons; Ref. 25). Transfection of trunks and whole embryos were done as controls. For luciferase assay, the heads (6–10) were homogenized and aliquots were assayed (using a protocol supplied by Promega) to determine the picograms of luciferase produced per well. The luciferase assay gave 50,000–100,000 relative light units/μg of protein (Sigma)/30 s using the single-channel luminometer (Berthold). Protein estimations were done using the Bradford reagent (Bio-Rad) and bovine serum albumin standards, and ranged from 10 to 20 μg/μl. Luciferase activity is reported as the mean (including all trials, n given in figure legend) ± S.E.

DNase Footprinting—In order to locate binding sites for potential transcription factors, DNase footprinting was performed on the rhodopsin proximal promoter. Using an adult Xenopus retinal extract, numerous extended regions were protected from digestion, including those predicted from sequence comparisons in the proximal promoter (Fig. 2A). At present, no Xenopus homologues of Crx, Nrl, or other rod-specific transcription factors have been identified. Therefore, footprinting of the proximal promoter was done using recombinant mammalian proteins purified from E. coli: the bovine Crx homodomain (GST-CrxHD; Ref. 16) and the DNA binding domain and surrounding bZIP regions of murine Nrl containing a hexahistidine tag (His-Nrl; Ref. 27). Both proteins protected the rhodopsin proximal region (Fig. 2, B and C). GST-CrxHD produced an extended footprint encompassing the Crx consensus sites from −153/−73 on the sense strand and −70/−156 on the antisense strand, respectively. Nrl protected a number of regions (−81/−56 on the sense strand and −57/−84 on the antisense strand) that included an AP1 consensus region and NRE, adjacent to two Crx sites. These results strongly suggest that the sequences in the Xenopus rhodopsin proximal promoter contain binding sites for members of the Otx2-related and neural retinal leucine zipper families.

Xenopus Rhodopsin Promoter: Analysis by Transfection of Xenopus Embryos—A comparison of promoter activity was performed using an improved transfection protocol (23), which allows direct comparison between the mean activities observed for different DNA constructs, without normalization with a second reporter plasmid. Initial experiments were per-
formed using three upstream fragments: (−5500/+41, −1300/+41, and −508/+41), which were all capable of driving tissue-specific expression in embryos (Table II). Comparative transfections performed at equimolar concentrations showed that the activity from the −5500/+41 fragment was −35% lower compared with the other two promoters, which did not differ, although this difference was not significant at p < 0.05. Size of the plasmid used in the transfection appeared to have little influence on reporter gene expression, as two derivatives containing additional sequences produced similar results (Table II). One of the derivatives contained the Xenopus rhodopsin gene and 3’ sequences, suggesting no significant transcriptional control regions downstream of the initiation region. Unlike that observed in heads, reporter expression from the upstream sequences in trunks was <2-fold over that obtained using GL2, which produced a relatively consistent but very low level of activity compared with heads transfected without DNA. We conclude that the major regulatory elements are located in the 508-bp proximal region.

To further characterize the transcriptional control sequences in the proximal promoter, transgenic tadpoles were generated (18, 33) using XOP(−508/+41) driving expression of GFP. GFP expression was found only in the eye and, transiently, in the pineal. In fixed sections of transgenic retina, GFP expression was observed only in rods (see below). Each rhodopsin-positive cell also expressed GFP, with expression apparent by stage 40 (data not shown). The levels of GFP expression for the three large upstream constructs were qualitatively very similar, confirming the results from the transfections: that there is apparently no significant difference between these constructs. Taken together, these results demonstrate that the immediate upstream rhodopsin sequences direct expression to the rod cells.

**Mapping the Rhodopsin Proximal Promoter**—To identify cis-acting elements in the XOP proximal promoter, a series of mutations in pXOP(−503/+41)luc were generated by either selective removal of nucleotides or by sequential DNA deletions from the 5′ end (Fig. 3). The 92-bp encompassing the transcription initiation site and including the TATA region were essential for activity, as deletion of this region from the 503-bp upstream fragment decreased luciferase expression 190-fold (Fig. 3). Activity from the −503/−46 fragment was equivalent to the promoterless control. However, the 92-bp region (−46/+41) in either orientation or as a tandem repeat gave background levels of expression, indicating that these nucleotides, although necessary, were not sufficient for transcription of reporter plasmids.

Analysis of luciferase activity from 5′ promoter deletions identified other sequences required for promoter function. All of the deletion constructs, except XOP(−44/+41), yielded luciferase activity significantly above that obtained using GL2, in transfections of embryo heads (Fig. 3). No significant differences in luciferase activity were observed when nucleotides −508 to −234 were deleted (Fig. 3), indicating that these nu-
nucleotides do not contribute significantly to the head-specific transcription from the −503/+41 rhodopsin promoter region. Deletion of the region spanning position −233 to −203 enhanced luciferase activity 2.4–2.8-fold over that observed using any of the four larger constructs (Fig. 3). No significant changes in activity compared with −202/+41 were obtained when nucleotides −202 to −171 were deleted. A drop in activity was observed with the deletion of −170 to −145, with activity comparable to that obtained using the full fragment, −508/+41. Thus, these results are consistent with a second regulatory region from −170 to −146. A further decrease in luciferase activity (4.5-fold) compared with XOP(−145/+41) was found when −145 to −128 was deleted. A 40-fold stimulation of luciferase expression from the −127/+41 fragment relative to that obtained using XOP(−44/+41) indicated that the sequence spanning positions −127 to −46 is the smallest fragment sufficient for promoter activity.

To test for tissue specificity, embryonic trunks were transfected using the various deletion constructs and activity was compared with the corresponding activity in head by normalization to total protein. When compared with the activity measured in heads, all trunk activities except that of XOP(−127/+41) were less than 4% of the head activity. Trunk activity from all the deletion constructs was slightly elevated compared with −503/+41 and were also higher than GL2, suggesting that some nonspecific, low level transcription may occur when nucleotides −503 to −330 are deleted. However, these results show predominant head-specific expression driven by sequences further upstream of proximal promoter sequences.

To further characterize the Xenopus rhodopsin proximal promoter, targeted disruptions were created to replace putative regulatory elements with a short linker sequence (Table I). To test if the selective disruption resulted in expression in nonretinal cells, the mutant constructs were simultaneously analyzed by measuring reporter activity in transfected heads and trunks. A disruption of the region from −233 to −203 of the Xenopus rhodopsin upstream sequence (XOP 4) resulted in a 3-fold increase in reporter gene expression in heads (Fig. 4). This increase in activity in heads did not result in any significant change in reporter activity measured in transfected trunks. These observations are consistent with the increase in activity associated with the 5′ deletion construct lacking the −233/−203 region. The removal of −170 to −146 (XOP 3) did not alter the expression levels in transfected heads or trunks (Fig. 4). Selective disruption of the Ret4-like core region and flanking nucleotides (−136 to −122) resulted in a 2–3-fold increase in luciferase expression in heads as compared with the wild type promoter. This increase in expression was not accompanied by any significant change in the levels of reporter expression in trunks. Alteration of one of the NRE/AP1-like sites (−120 to −109) did not significantly affect the head-specific expression of the rhodopsin promoter or lead to any change in activity in trunks. However, disruption of the second NRE-like site (−84 to −58) dramatically reduced expression (Fig. 4). This was not caused by a change in spacing since an additional construct in which the spacing of the wild type rhodopsin promoter was maintained by addition of a short insert of random sequence was also significantly lower than the −503/+41 promoter construct. These results indicate an essential role for this NRE-like element in maintenance of high level expression of rhodopsin in Xenopus rod cells. A change in either one (−98 to −91) or both (−107/−91) of the GATTA sequences caused a small reduction in expression of the transgene 20% or 32%, respectively, that was not statistically significant. Finally, replacement of nucleotides −52 to −38, corresponding to the conserved Ret4 region in the bovine rhodopsin upstream sequence, did not change either the reporter luciferase levels or head-specific expression from the wild type promoter.

Role of the Xenopus Rhodopsin Basal Element—To address the contribution of the basal region in determining the specificity of expression from the Xenopus rhodopsin promoter, the sequences encoding the TATA region, initiation site, and sur-
GFP fluorescence, which was restricted to the eye. Animals

Transgenic Xenopus—To investigate the cellular expression patterns from the altered rhodopsin promoters, transgenic Xenopus were generated with the various deletion constructs driving expression of GFP. Several transgenic lines were generated for each construct, and GFP expression was analyzed in tadpoles. Transgenic animals harboring deletions of (−233/−203) and (−136/−122) exhibited significant enhancement of GFP fluorescence, which was restricted to the eye. Animals

FIG. 5. Transient transfections of Xenopus embryos with XOP basal element replacement constructs. A, the basal region of the Xenopus rhodopsin proximal promoter (−36/+41) was replaced with either the corresponding basal region from Xenopus cardiac actin (XOP-CAR, −36/+36) or the CMV basal sequence (XOP-CMV, −36/+25). 

B, sequence comparison of the Xenopus rhodopsin, cardiac actin, and CMV promoter basal sequences show no discernible homology in the sequences replaced. The dark shaded boxes indicate the TATA region and the transcriptional start site. The sequences in the gray shaded box include the conserved Ret 4-core sequence that was included while synthesizing the XOP-CAR and XOP-CMV constructs. C, luciferase activity normalized to the wild type promoter (WT) from four to six independent transfection experiments using heads or trunks was plotted. Activity levels from the wild type cardiac actin construct and CAR and CMV promoter in heads and trunks are shown for comparison.

rounding nucleotides were tested. The XOP basal region comprising nucleotides −36/+41 was replaced by basal element regions from two non-retina-specific promoters, Xenopus cardiac actin (XOP-CAR, −36/+36; Ref 20) and CMV (−36/+25) promoters (Fig. 5A). These basal regions do not possess any obvious sequence homology to the XOP basal region (Fig. 5B). Replacement of the XOP basal element with either cardiac actin or CMV basal region did not significantly affect the expression levels of the hybrid constructs as compared with wild type rhodopsin in transfected heads or trunks (Fig. 5C). These results show that the heterologous basal regions can functionally substitute for the XOP basal region and that the upstream cis-acting sequences are capable of controlling the cell specificity of rhodopsin expression.

Transgenic Xenopus—To investigate the cellular expression patterns from the altered rhodopsin promoters, transgenic Xenopus were generated with the various deletion constructs driving expression of GFP. Several transgenic lines were generated for each construct, and GFP expression was analyzed in tadpoles. Transgenic animals harboring deletions of (−233/−203) and (−136/−122) exhibited significant enhancement of GFP fluorescence, which was restricted to the eye. Animals
The Xenopus rhodopsin promoter contains a number of potential Otx2-like binding sites (TAAT or ATTA) between −503 to −72. A GATTA repeat, previously identified as BAT-1, shows a high degree of conservation among the opsin promoters (40) as well as other photoreceptor-specific genes (41). The BAT1 region in the bovine opsin promoter has been shown to be a binding site for HMG I(Y) (29, 42) and to contribute to the promoter activities in transfected cells, but not in vitro transcription assays (28). Surprisingly, targeted disruption of this region in the Xenopus promotor caused no significant change in either transcriptional levels or pattern of expression (Figs. 4 and 6). Moreover, the presence of a GATTA sequence upstream of the basal promoter was not sufficient to support a high level of rod-specific expression in transgenic Xenopus (Fig. 7). The GATTA repeat may serve different functions in the different photoreceptor gene promoters. In the case of the IRBP promoter, which drives expression in both rods and cones, deletion of the GATTA element dramatically reduced transcription in transfected chick retinal cultures (43) and transgenic mice (44). In this case, the Ret-1 GAATTA site was not sufficient to overcome the mutations in the GATTA sequence. Due to the high degree of sequence conservation across species, it is tempting to propose that the GATTA region in XOP does have a functional role. Our inability to detect a significant effect in the BAT-1 mutation experiments (Figs. 4 and 6) could be explained by the presence of redundant elements in the proximal promoter.

Targeted disruption of the conserved cis-acting element, Ret-1/PCE I in the Xenopus opsin promotor resulted in a 2-fold increase in transcriptional activity in transfected retina and increased GFP expression in transgenic animals. The discrepancy between the results obtained in the 5´ deletion and targeted disruptions indicates that the regulatory properties of the Ret-1 element may be determined by upstream cis-acting sequences, perhaps through interactions with transcription factors bound to these elements. This supports our results obtained using synthetic promotor constructs, containing one or more copies of the Ret-1 region (−144 to −120) upstream of the rhodopsin basal region (−51/+41), which contribute to 0.1% or less of the Xenopus rhodopsin promotor’s transcriptional activity in transient transfections (data not shown). Several proteins have been shown to bind to the Ret-1/PCE I sequence including Crx (16), Rx (31), and Erx (17), each of which functions as a weak transcriptional activator in transfection assays. However, these proteins most likely interact with other retinal transcription factors, as seen in the case of a direct interaction between Nrl and Crx (16, 45), resulting in synergistic activation of the bovine opsin promoter. It is unclear if the spacing of the Ret-1 sequence relative to the transcriptional start site affects its regulatory role. The targeted disruptions may have resulted in a promoter conformation more favorable to the assembly of transcription factors, thereby stimulating the transcriptional activity.

The region between −170 to −146 (XOP 3) and −233 and −203 (XOP 4) contains sequences that were footprinted with adult Xenopus nuclear extracts (Fig. 2A). In retinal transfections, deletion of −233 to −203 caused a 3-fold increase in transcription that was also seen in transgenic animals, suggesting that this region contains a negative regulatory element. The sequences in the XOP 4 region do not possess any sequence similarity to the mammalian opsin promotores or a recognizable consensus transcription factor binding site. Although there is evidence for a negative regulatory region in mammalian opsin
promoters, the sequences do not share any similarity with XOP 4. There is limited sequence similarity between the Xenopus and mammalian promoters in the XOP 3, only encompassing an E-box motif, CANNTG at nucleotide –163 to –158. In the mouse opsin promoter, the equivalent E-box sequences were shown to bind MASH-1 using mobility shift assays (46). The Xenopus homologues of MASH proteins (XASH-1 and XASH-3) are only expressed in the ciliary margins in the laminated retina (47) and not in the photoreceptors. Therefore, if XOP 3 function is regulated by its conserved E-box motif, then either a different class of bHLH proteins bind to this region in the Xenopus opsin promoter, or the effect of XOP 3 on opsin expression is exerted at a step prior to photoreceptor differentiation. Targeted disruption of these sequences, however, showed no change in transfected retina but greatly reduced levels of reporter expression in transgenic animals. The discrepancy between the two assays for this construct suggests that protein binding to XOP 3 deletion may be sensitive to the chromatin environment or the proteins that bind to XOP 3 may be part of the chromatin-remodeling complex. This region contains an AT-rich sequences (at least five consecutive AT at –152 to –147). Further experiments are needed to determine if HMG I(Y) actually binds to the xenopus proximal promoter and activates transcription via any of these sequences.

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FIG. 7. The BAT 1 and NRE sequences in an artificial XOP promoter construct drives GFP expression in the developing central nervous system. A, schematic representation of an artificial promoter construct containing wild type XOP promoter sequences from –96 to +41 driving expression of the GFP reporter gene. The construct includes the 3’ GATTATA sequence of the BATI region (–96/–89), the NRE consensus sequence TG(N)8GC (–73/–62) denoted by boxes, and the basal promoter region (–61/+41). The sequences upstream of –96 include a short (6 bp) random linker used for cloning. B, developmental time course of GFP expression in transgenic tadpoles generated using the –96/+41 GFP construct. Transgenic tadpoles were produced and analyzed for GFP expression from 3 to 7 days post injection (dpi). Bright field and fluorescent images of tadpoles generated with –96/41GFP (top panel) showing GFP expression in eye, brain, and spinal cord 4–7 days after injection. The background auto fluorescence from the yolk is seen in developing tadpoles injected with either –96/41 XOP-GFP or sperm nuclei alone (nontransgenic controls, lower panels).
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