Xenopus Brn-3.0, a POU-Domain Gene Expressed in the Developing Retina and Tectum
Not Regulated by Innervation

Nicolas Hirsch and William A. Harris

Purpose. To study the effect of denervation on the expression of the POU-domain gene Brn-3.0 in the Xenopus visual system.

Methods. An oligonucleotide probe was used to identify homologs of the murine gene Brn-3.0 in the retina. In situ hybridization was used to determine the spatial distribution of the mRNA within the developing embryo. To study the effects of denervation on Brn-3.0 expression and cell fate, embryonic eyes were transplanted to an ectopic location on the animal (the flank) before the onset of retinal ganglion cell (RGC) axonogenesis. Gene expression in ectopic eyes and denervated tecta was analyzed over time using in situ hybridization.

Results. The deduced, partial amino acid sequence for Xenopus Brn-3.0 shows 100% identity with the mouse Brn-3.0 and the human Brn-3a gene products. It is expressed during early embryonic development in distinct populations of the neural crest and later in specific cranial ganglia. It also is expressed in RGCs and in the optic tectum, beginning before the first RGC axons have reached the tectum and continuing without interruption throughout the period when retino-tectal connections are established and refined. If the retino-tectal projection is kept from forming by transplanting one eye to an ectopic location, Brn-3.0 expression is unaffected in both the ectopic eye and the denervated side of the tectum.

Conclusions. Coordinated expression of Brn-3.0 in afferent and efferent pathways suggests mutual regulation. However, the authors’ evidence shows that expression in the retina is not regulated by target-derived factors nor is expression in the tectum regulated by retinal innervation. Invest Ophthalmol Vis Sci. 1997; 38:960-969.

The principal task faced by projection neurons on reaching a target is to make and maintain the appropriate synaptic connections. This can be an especially complex problem when the afferent neuron and its target are in different environments. The projection of retinal ganglion cell (RGC) axons to the optic tectum is a particularly well-studied example. Although much is known about cell surface receptors and signal transduction cascades that function in this process, less is known about the nuclear factors that regulate expression of these cell surface receptors and activate gene expression in response to the transduction of extracellular signals.

One potentially interesting family of nuclear proteins are the POU-domain transcription factors, which are expressed widely in the developing nervous system. The POU-domain is a bipartite DNA-binding region that can activate transcription both in vitro and in vivo. Loss of function mutations in POU-domain proteins result in the failure of specific populations of cells to differentiate and express cell type-specific genes. A large subfamily of POU-domain genes, the Brn (Brain) genes, are expressed throughout the central nervous system during embryonic development in the mouse. Brn-3.0, -3.1, and -3.2 are three murine genes that are expressed in subsets of RGCs as well as in the superior colliculus, the mammalian homolog of the optic tectum. This pattern suggests a possible role...
in the formation or maintenance or both of retinotectal connections. Homologous genes are found in humans and are designated Brn-3a, -3c, and -3b, respectively.14,15 These genes all have a high degree of sequence similarity in the POU-specific and POU-homeodomains, and their spatial patterns of expression in the embryo overlap to a significant degree. Mice in which the Brn-3.2 gene has been deleted show a significant loss of RGCs and a disruption in the morphology of the superior colliculus.17 In addition, the Brn-3a (Brn-3.0) gene product activates the promoter of the neuronal intermediate filament protein a-interneixin18 and the promoter of SNAP-25, a protein involved in neurite outgrowth in vitro.19 Cell lines expressing antisense Brn-3a RNA showed decreased levels of SNAP-25 mRNA and reduced neurite outgrowth.20 These results support the hypothesis of a role for Brn-3 family members in synaptogenesis and map formation during tectal development.

Given the extensive body of work detailing the processes of pathfinding, synaptogenesis, and topographic mapping in the retina–tectal system of Xenopus laevis, the identification of nuclear factors expressed in the retina and tectum is a significant step toward understanding how these processes work. Here we report the identification of a member of the Brn-3 gene family in this species. Detailed analysis of the sequence and expression pattern confirms that it is a homolog of the mouse Brn-3.0 and the human Brn-3a genes. Xenopus Brn-3.0 is expressed at high levels in the retina and optic tectum throughout pathfinding, target recognition, and synaptogenesis. If the connection between the retina and tectum fails to develop early in development, however, the levels of Brn-3.0 mRNA do not change. So, although this gene may play a role in the development of the retina–tectal system, we have shown that normal synaptic connections between retina and tectum do not regulate its expression.

MATERIALS AND METHODS

Cloning and Sequencing

A 1024-fold degenerate oligonucleotide probe was designed to hybridize with the amino acid sequence WFCNRRQK, a highly conserved region in helix 3 of the POU-homeodomain thought to interact with the major groove of the DNA molecule.21 The nucleotide sequence of this probe is 5'-TT(TC) TG(AGTC) G(GT)(AGTC) C(GT)(AG) TT(AG) CA(AG) AAC CA-3'. It was synthesized (Applied Biosystems, Foster City, CA) and deprotected in 11.5 M ammonium hydroxide at 55°C for 16 hours. This probe was used to screen a randomly primed Xenopus metamorphic tadpole cDNA eye library in Agt10, which was plated at a density of 6 x 10⁴ plaques/plate on 25 plates for a total of 1.5 x 10⁷ recombinant plaques. The oligonucleotide was 5’ end labeled with 7000 Ci/mmol ³²P (ICN, Costa Mesa, CA) using polynucleotide kinase (New England Biolabs, Beverly, MA). Duplex nitrocellulose filters were made from the plated phage.22 These filters were prehybridized for 12 hours at 37°C in 6X SSC, 5X Denhardt’s, 0.05% sodium pyrophosphate, 1% sodium dodecyl sulfate, and 0.1 mg/ml of sheared, denatured salmon sperm DNA. Filters then were hybridized in prehybridization buffer containing 200 pmol of oligonucleotide probe for 24 hours at 42°C. Filters were washed three times for 10 minutes each in 6X SSC, 0.05% sodium pyrophosphate at room temperature, and then twice for 20 minutes each in 3 M Me₃NCl, 0.05 M Tris at 55°C. This washing step was added to eliminate any hybrids that had more than two single-base mismatches,23 thus making it specific for cDNAs containing only the POU-type homeodomain.

Two of the most strongly hybridizing clones, designated XEH (Xenopus eye homeobox) 2.3 and 6.1, were plaque purified and subcloned into the EcoRI site of the pBSII(SK+) plasmid (Stratagene, La Jolla, CA). Both clones were sequenced completely by the deoxy chain termination method.24 Sequencing was carried out using either the Sequenase 2.0 (United States Biochemicals, Amersham, Cleveland, OH) or Vent DNA polymerase Thermal Sequencing kit (New England Biolabs) following the manufacturer’s instructions. DNA sequences were analyzed using the Intelligenetics DNA Analysis software package (Intelli- genetics, Palo Alto, CA). Deletions of clone XEH 2.3 (hereafter referred to as Xenopus Brn-3.0) were made using the Erase-a-Base kit (Promega).

In Situ Hybridization

Albino Xenopus laevis embryos were obtained from pair matings of animals raised at the University of California, San Diego. Investigations adhered to the ARVO Statement for the Use of Animals in Ophthalmic Vision Research. Embryos were dejellied with 2% cysteine, pH 8, in 10% Holtfreter’s solution for 2 to 3 minutes and then washed 10 times in 10% Holtfreter’s. They then were stained with 0.5% Nile Blue for 5 minutes and washed three times in 10% Holtfreter’s. All embryos were staged according to Nieuwkoop and Faber25 and were maintained at either 14°C or 21°C before fixation. Embryos were fixed in MEMFA (0.1 M MOPS, 2 mM ethylenediaminetetraacetic acid, 1 mM magnesium sulfate, 3.7% formaldehyde) for 2 hours at room temperature and stored in ethanol at −20°C. The expression pattern of Xenopus Brn-3.0 mRNA in the developing embryo was assayed by in
situ hybridization. The entire length of the *Xenopus* Brn-3.0 cDNA, including 1.6 kb of 3' untranslated sequence, was used to make a sense template using the restriction endonuclease Hind III and an antisense template using the restriction endonuclease BamHI. A digoxigenin-labeled RNA probe was synthesized from these templates using T3 RNA polymerase for sense probe and T7 RNA polymerase for antisense probe (all enzymes supplied by Boehringer Mannheim, Mannheim, Germany). Wholemount in situ hybridization was carried out as described previously. Whole embryos were postfixed in MEMFA overnight at 4°C, dehydrated in graded ethanols, and cleared in 2:1 benzyl benzoate/benzyl alcohol. Wholemount embryos were photographed in benzyl benzoate/benzyl alcohol on a Zeiss (Oberkochen, Germany) Axioskop. Darkly stained embryos were selected for paraffin sectioning. These embryos were dehydrated in graded ethanols, cleared in xylene (2, 10 minute changes) at room temperature, then infiltrated for 10 minutes in 1:1 xylene:paraplast and three times for 1 hour each in paraplast (Oxford, Wilmington, NC) at 60°C before embedding and sectioning. Ten-micron sections were cut on a Leitz rotary microtome, dried onto Meyer's albumin-coated slides, dewaxed (twice for 10 minutes in xylene), and coverslipped in Permount (Fischer Scientific, Pittsburgh, PA) before photomicrography.

Identification of brain regions expressing Brn-3.0 was performed using anatomic charts published previously of the frog brain.

**RESULTS**

*Xenopus* Brn-3.0 is a Homolog of Murine Brn-3.0 and Human Brn-3α

Of the cDNAs found by this screen, XEH 6.1 was determined to be a full-length clone of XLPOU3, a *Xenopus* POU-domain gene cloned previously. In situ hybridization experiments show expression of XLPOU3 throughout the brain beginning at stage 24, but no expression is detected in the developing eye (not shown). A 2-kb clone designated XEH 2.3 was found to be homologous to portions of the murine gene Brn-3.0. This clone was selected for further study and designated *Xenopus* Brn-3.0.

*Xenopus* Brn-3.0 (Brn-3.0) encodes a partial cDNA with its 5' end beginning at amino acid position 318 within the POU-specific domain and containing the entire POU-homeobox and a translation stop codon, as well as approximately 1.6 kb of 3' untranslated sequence. The coding region of Brn-3.0 is 104 amino acids in length, or approximately 25% of its murine and human homologs. Repeated attempts to clone a full-length Brn-3.0 cDNA were unsuccessful. This was most likely caused by a highly G + C rich region of sequence immediately upstream of the POU-specific domain inhibiting reverse transcription and reducing significantly the percentage of full-length cDNAs in the library.

The deduced amino acid sequence of *Xenopus* Brn-3.0 is 100% identical to that of mouse Brn-3.0 and human Brn-3α. Although *Xenopus* Brn-3.0 also is 99% identical to mouse Brn-3.1 and Brn-3.2 and human Brn-3b and Brn-3c in the POU-specific and POU-
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FIGURE 2. Expression of *Xenopus* Brn-3.0 mRNA in the brain, spinal cord, and neural crest. In all panels, dorsal is up. In A, D, and E, anterior is to the right. (A) A lateral view of a stage 26 *Xenopus* embryo. The arrows indicate migrating cells of the mandibular crest segment (MCS) that stain for *Xenopus* Brn-3.0. The arrowhead indicates cells of the hyoid crest segment just beginning their migration. Lines labeled B and C indicate the approximate plane of section in B and C, respectively. (B) A cross-section just posterior to the eye at stage 26. Arrows indicate a portion of the MCS as it migrates both dorsally and ventrally to the eye. (C) A cross-section through the spinal cord of the same embryo in B. Brn-3.0 expression in the spinal cord is confined to a discrete population of interneurons (arrow). This staining persists through stage 42, although the most intensely stained cells are located further posteriorly as the embryo grows. (D) A lateral view of a stage 33/4 embryo. Asterisks identify developing cranial ganglia. Arrows show the simultaneous onset of expression in the retina and optic tectum. Arrowhead shows the otic vesicle that has high levels of background hybridization. (E) A dorsolateral (V4) view of a stage 42 tadpole. This view best illustrates the pattern of *Xenopus* Brn-3.0 expression in the mature brain. The three arrows show a stripe in the met-meyelencephalon (M), in the tectal staining in the mesencephalon (T), and in the dorsal diencephalon (D). Arrow with R indicates the retinal ganglion cells staining for Brn-3.0. The dark line at the outer edge of the eye is the retinal pigment epithelium. Asterisks identify cranial ganglia as in D. Scale bar = 200 μm (A,D,E), = 100 μm (B,C).

homeodomains, it differs significantly from them in the 16 amino acid linker between the 2 regions and in the 7 amino acid stretch at the carboxy-terminal end of the protein (Fig. 1). *Xenopus* Brn-3.0 also has a valine (V) at position 23 (amino acid 378 in Fig. 1) of the homeodomain, as do Brn-3.0 and 3a, whereas Brn-3.1, -3.2, -3b, and -3c all have an isoleucine (I) at that position.

Expression of *Xenopus* Brn-3.0 mRNA in Brain and Neural Crest

The first detectable *Xenopus* Brn-3.0 expression can be seen, using in situ hybridization, at stage 23 in a few unidentified cells in the developing spinal cord. No significant staining was seen using the sense probe as a control, except in the otic vesicle and brain ventricles at later stages. By stage 26, streams of migrating neural crest cells can be seen in whole mount, both anterior to and posterior to the developing eyebud (Fig. 2A). More Brn-3.0-expressing cells can be seen ventral to the eye, apparently continuing a line extending from the neural tube to the pharynx (arrow in Fig. 2A), but this ventral component of expression is not detectable at later stages. The neural crest cells can be seen in a cross section through a stage 26 embryo just posterior to the eye (Fig. 2B). They appear to be discrete clusters of cells in the mesenchyme surrounding the eyebud. Also at this stage, Brn-3.0 expression can be seen in defined groups of cells in the mediolateral spinal cord that occur at regular intervals.
along its length, beginning just posterior to the hindbrain–spinal cord boundary (Figs. 2A and 2C).

By stage 33/4, the most ventral streams of cells seen at stage 26 have stopped expressing Brn-3.0. More posteriorly, however, the developing cranial ganglia express it at high levels (asterisks in Figs. 2D and 2E). The onset of expression in the retina, optic tectum (dorsal midbrain), and forebrain also is apparent at this stage. Expression in the spinal cord continues, although at lower levels relative to stage 26. By stage 42, the mature staining pattern of Brn-3.0 is apparent and changes little during the remainder of embryonic development (Fig. 2E). In the brain, expression is seen in the met–meyelencephalon, mesencephalon (tectum), and dorsal diencephalon. It also is expressed in the RGCs and in several cranial ganglia.

Expression of *Xenopus* Brn-3.0 mRNA in Retina and Tectum

In *Xenopus*, the first cells in the retina to become postmitotic are in the ganglion cell layer beginning at stage 24.33 *Xenopus* Brn-3.0 mRNA expression is not detectable in the ganglion cells until 16 hours later, at stage 35/4 (Fig. 3A). Brn-3.0 also is expressed in the optic tectum, the target of the RGC axons. The tectal expression is first detectable at stage 33/4, coincident with the onset of expression in the RGCs. Brn-3.0 mRNA is found throughout the dorsalventral extent of the tectum, although it is absent from the mitotic cells in the ventricular layer (Fig. 3B). Staining at the ventricular surface (arrow in Fig. 3D, also seen in Fig. 3B), however, is an artifact of nonspecific sticking of the digoxigenin-labeled probe to the lining of the ventricle (V). This nonspecific staining is most prominent in embryos hybridized in whole mount and is not observed in tissue sections hybridized on a glass slide, such as those in Figure 4A and 4B. It also is excluded from the dorsal-most part of the neural tube throughout embryonic development (arrowheads in Figs. 3B, 3D, and 3G). By stage 42, more than 99% of the cells in the central retina have become postmitotic.35 *Xenopus* Brn-3.0 staining is visible at this stage in the ganglion cell (G) layer, but not in the cells of the inner nuclear (I) layer or the outer nuclear (O) layer. Nor can any Brn-3.0 expression be detected in the undifferentiated cells of the ciliary marginal zone (outlined in Figs. 3A and 3C), which is a population of dividing undifferentiated cells that persists throughout the life of the animal and serves as a source of new cells as the retina grows.34 The tectal staining pattern remains largely unchanged through stage 42, although at this point staining can be seen almost up to the ventricular surface (Fig. 3D).

The progress of the retina–tectal projection from stage 33/4 to stage 42 is shown schematically in Figure 3, panel 3E.35 The expression of *Xenopus* Brn-3.0 mRNA begins in the RGCs and the tectum 10 hours before the RGC axons reach their target (development occurring at room temperature). By stage 42, most of the axons from the central retina have reached the tectum and have begun to form synapses with the tectal cells. The pattern of Brn-3.0 expression is unchanged in the retina at stage 50, 14 days after it first appears (Fig. 3F). Brn-3.0 message in the tectum still can be detected at stage 50 (Fig. 3G), long after the RGC axons have made synaptic connections with the proper targets. At this stage, it is evident that the mRNA is localized to the cellular layers, and not the neuropil.

*Xenopus* Brn-3.0 Expression Is Not Regulated by Tectal Innervation

In *Xenopus*, the first RGC axons grow out of the eye at stage 31, making their earliest contact with target cells in the contralateral tectum at stage 37/8.36,37 All of the axons from the central retina have reached the tectum and made synaptic connections with their targets by stage 45. Given the remarkable conservation of the retinal and tectal staining patterns between *Xenopus* and mouse, we wanted to determine if *Xenopus* Brn-3.0 expression still could be detected in these tissues in the absence of their usual synaptic connections. To test this, one eye from a stage 24 embryo was transplanted to the flank endoderm, preventing the RGC axons from finding their target. Previous work has shown that the transplanted eye will develop normally in this ectopic location and that ganglion cell fate, as determined from morphology, is unaffected.38 However, our analysis shows that the number of ganglion cells in the ectopic eye decreases to approximately 65% of control by stage 50. Embryos were allowed to grow to either stage 35/4, 45, or 50 before fixation and in situ hybridization.

*Xenopus* Brn-3.0 expression in both the retina and the tectum was found to be independent of innervation at all stages studied. Tectal expression of Brn-3.0 is unaffected by removal of either one or both eyes. In Figure 4A, the right eye of a stage 33/4 embryo has been removed at stage 24. The pattern of Brn-3.0 expression is identical in both sides of the tectum and is indistinguishable from tecta not operated on, which is seen in Figure 3G. This result is to be expected, however, because normal innervation of the tectum would not occur until stage 37/8 at the earliest. The denervated half shows no change in Brn-3.0 expression at stage 50 (Fig. 4B), approximately 13 days after it normally would have received retinal input. In the retina, Brn-3.0 is confined to the ganglion cell (G) layer in both control and ectopic eyes. The onset of expression is coincident at stage 33/4 (Figs. 4C and
FIGURE 3. *Xenopus* Brn-3.0 mRNA expression in the developing retina and tectum. In all panels, dorsal is up. In A, C, and F, the epidermal surface is to the right. A, C, and F show cross-sections of the eye in embryos at stages 33/4, 42, and 50 respectively. The staining is confined to the ganglion cell layer in all cases. The ciliary marginal zone (outlined in A and C, arrow in F) clearly is devoid of staining. B, D, and G show cross-sections of the optic tectum at the same stages. Note that *Xenopus* Brn-3.0 expression is not detected at the most dorsal part of the brain (arrowhead) nor is it present in the ventricular zone. The staining at the ventricular (V) surface in D (arrow) and also seen in B is artifactual. E shows the progress of the retinotectal projection between stage 33/4 (seen in A and B) and stage 42 (seen in C and D). Note that at the onset of *Xenopus* Brn-3.0 expression, the earliest retinal ganglion cell axons have just begun to turn dorsally and will not reach the tectum until stage 37/8. By stage 42, most axons have reached the tectum and have started to branch.

G = retinal ganglion cell layer; I = inner nuclear layer; O = outer nuclear layer; NB = neuroblastic layer; L = lens; R = retina; T = optic tectum. Scale bar = 150 μm.

4D) and persists unchanged until stage 50 (Figs. 4E and 4F). This result was seen in 10 transplants at stage 33/4 and in 11 transplants at stage 50 and was repeated in 2 separate experiments. Although staining in the ganglion cell layer in the ectopic eye may appear more intense, that is an artifact of the compression of the remaining ganglion cells into a smaller area because the ectopic eye usually is reduced in size relative to control eyes. *Xenopus* Pax-6 is used as a second marker for ganglion andamacrine cells in the ectopic eye, and its expression similarly is unaffected (Figs. 4G and 4H).

**DISCUSSION**

Members of the POU family of transcription factors play important roles in the development and differentiation of specific cell types. Many of these genes are expressed in neural tissues at particular times in development when cell fate decisions are being made, including the Brn-3.
**FIGURE 4.** *Xenopus* Brn-3.0 expression in the retina and tectum is independent of innervation. In all panels, dorsal is up. In C to H, the epidermal surface is to the right. (A,B) Cross-sections through tecta from embryos at stages 33/4 and 50, respectively, that have had the right eye transplanted to the flank at stage 24. The side of the tectum that is not innervated is indicated by an arrow in both . In A, the control eye is visible at the bottom right. E = control eye; Np = neuropil. (C to H) Control (top row) and transplanted (bottom row, ectopic) eyes assayed for *Xenopus* Brn-3.0 expression at the stages indicated. (C,D) The onset of Brn-3.0 staining is not affected by transplantation and is coincident with the onset in embryos not operated on seen in Figure 3A. (E,F) Brn-3.0 is expressed continually in retinal ganglion cells in the absence of normal tectal innervation until at least stage 50. (G,H) A second marker for ganglion cells, Pax-6, is expressed at equivalent levels in control and ectopic eyes. Pax-6 also is expressed in the amacrine cells of the inner nuclear layer. G = retinal ganglion cells; I = inner nuclear layer; L = lens. Scale bar = 100 μm.
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subfamily, which is expressed in the developing retina and tectum at a time when retinal and tectal neurons are establishing and refining their synaptic connections. We report here the cloning of a *Xenopus* homolog of the murine and human Brn-3 genes. *Xenopus* Brn-3.0 is expressed in the RGCs after they have become postmitotic, in a pattern similar to Brn-3.2. However, it also is expressed earlier in development in streams of apparently undifferentiated neural crest cells. This pattern is more suggestive of Brn-3.0 than Brn-3.2, which is not expressed at all in mitotic cells.11

The amino acid sequence comparison between our partial clone of *Xenopus* Brn-3.0 and the other Brn family members shows it to be related most closely to murine Brn-3.0 and human Brn-3a. All three share 100% identity, even in the variable linker region between the POU-specific and POU-homeodomains, a region that is characteristic of a particular class of POU-domain.10 Additional evidence is provided by the sequence of the Brn-3.0 POU-homeodomain, which has a valine (V) residue at position 23, whereas both Brn-3.1 (3c) and 3.2 (3b) have an isoleucine (I) at this position. Single amino acid changes in this region have been shown to be sufficient to change the homeodomain’s DNA binding specificity.39 This difference also is significant in distinguishing *Xenopus* Brn-3.0 from a Brn-3 family member cloned previously in *Xenopus*.40 The polymerase chain reaction fragment identified in that case has an isoleucine (I) at position 23 of the homeodomain and is therefore a homolog of either Brn-3.1 or -3.2.

*Xenopus* Brn-3.0 mRNA is detected beginning at approximately 24 hours of development (stage 23). Its earliest expression is in segmentally repeated groups of cells in the developing spinal cord, which, based on their position and on the stage at which they appear, may be commissural interneurons.41 At stage 26, Brn-3.0 is expressed in streams of cells stretching from the dorsal-most part of the neural tube to the pharynx. These cells are in the same position at this stage of development as are the neural crest cells of the mandibular crest segment and hyoid crest segment, which migrate from the dorsal mesencephalon to the mandibular and hyoid arches of the pharynx, respectively.42 Brn-3 genes also are expressed in the migrating neural crest and the cranial ganglia of the chicken.43 Although Brn-3.0 expression in the neural crest has diminished by stage 33/4, it clearly is present in the developing cranial ganglia. This pattern of expression is similar to that of the mouse, where Brn-3.0 is found in the trigeminal ganglion and in the vagus complex.35 Expression in the brain at stage 42, called the mature pattern because it continues unchanged to stage 50 (12 days later), can be divided into three parts. First, in the doroanterior diencephalon, Brn-3.0 is expressed at high levels in the habenula. Second, in the mesencephalon, it is expressed in the optic tectum and in a discrete region of the tegmentum. Third, in the myelencephalon, it is expressed in a cluster of unidentified neurons. Here, too, there are similarities with the mouse. Murine Brn-3.0 is expressed uniquely at high levels in the habenula and in the tegmentum, and both Brn-3.0 and Brn-3.2 are expressed in the mammalian equivalent of the optic tectum, the superior colliculus.11,13

*Xenopus* Brn-3.0 is expressed in the developing retina and optic tectum simultaneously at stage 35/4, 10 hours before the first RGC axons arrive. It continues to be expressed during the time when connections between RGC axons and tectal target cells are being elaborated and refined. This pattern is highly suggestive of *Xenopus* Brn-3.0 playing a role in developing the synaptic connections between these two cell types. However, the levels of expression do not decline after the synaptic connections have been established, suggesting a possible role for this gene in maintaining the connections once they have been made. Knockouts of a Brn-3.0 related gene, Brn-3.2, in the mouse exhibit a significant decline in RGC number44 and a disruption in the normal morphology of the superior colliculus.17 This evidence strengthens our argument, given that both Brn genes are expressed in the retina and tectum and may play redundant or complementary roles in making and maintaining synaptic connections.

The independent regulation of *Xenopus* Brn-3.0 expression in both retinal and tectal cells that have been deprived of their synaptic connections shows that the onset and maintenance of *Xenopus* Brn-3.0 does not rely on contact between the RGCs and their target cells. From stage 33/4, when Brn-3.0 is first expressed, through stage 50, mRNA levels in ectopic eyes and in denervated tecta appear little changed when measured by in situ hybridization, even though there is a decline in the number of RGCs by 35% over this period. Recent work in chick and in rat has shown that the loss of RGCs in axotomized retinas can be reduced or even prevented by intraocular injection of the neurotrophins brain-derived neurotrophic factor or NT-4.45-48 Our results imply that at least some Brn-3.0 expressing cells in the retina do not require tectally derived trophic factors to survive. Although this provides strong evidence that *Xenopus* Brn-3.0 expression is not regulated by innervation, it still may regulate downstream events that are involved in pathfinding, target recognition, or synaptogenesis in the retinotectal system. Further experiments are needed to study the optic pathway in Brn-5 knockout animals and to determine which downstream genes, if any, still function in vivo.
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References


