Developmental Regulation of CPG15 Expression in Xenopus

ELLY NEDIVI,1 ASHKAN JAVAHERIAN,2 ISABEL CANTALLOPS,1 AND HOLLIS T. CLINE1*

1Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724
2Graduate Program in Genetics, State University of New York at Stony Brook, Stony Brook, New York 11790

ABSTRACT
Mechanisms controlling dendritic arbor formation affect the establishment of neuronal circuits. Candidate plasticity gene 15 (CPG15) is a glycosylphosphatidyl inositol (GPI)-linked activity-induced protein that has been shown to function as an intercellular signaling molecule that can promote the morphological and physiological development of the Xenopus retinotectal system. A thorough understanding of CPG15 function requires knowledge of the spatiotemporal expression of the endogenous protein. We therefore cloned Xenopus cpg15 and used RNA in situ hybridization and immunohistochemistry to determine the pattern of CPG15 expression. cpg15 mRNA and CPG15 protein are first detectable in the developing spinal cord and become widespread as development proceeds. CPG15 is expressed in sensory regions of the brain, including the visual, auditory, and olfactory systems. Within the retina, CPG15 is only expressed in retinal ganglion cells. CPG15 protein is concentrated in axon tracts, including retinal axons. These data support a model in which CPG15 expressed in retinal ganglion cells is trafficked to retinal axons, where it modulates postsynaptic dendritic arbor elaboration, and synaptic maturation. J. Comp. Neurol. 435:464–473, 2001. © 2001 Wiley-Liss, Inc.

Indexing terms: neuritin; ligand; retinotectal; GPI; spinal cord; amphibian; plasticity; visual system

Central nervous system (CNS) development is characterized by the coordinate development of presynaptic axonal arbors, postsynaptic dendritic arbors, and synaptic connections between them. Activity-dependent mechanisms regulate multiple aspects of nervous system connectivity during development. One way that activity can influence the development of neuronal connections is through the induction of activity-regulated genes, or candidate plasticity genes (CPGs). Numerous activity-induced genes have been identified through the use of highly selective screens (Nedivi et al., 1993; Qian et al., 1993; Grant sponsor: NIH; Grant sponsor: the National Down Syndrome Society; Grant sponsor: the Ellison Medical Foundation. Elly Nedivi and Ashkan Javaherian contributed equally to this article. Elly Nedivi’s present address is the Center for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, 50 Ames St., E18-670, Cambridge, MA. 02139.

CPG15, also known as neuritin (Naeve et al., 1997), is a small secreted protein, anchored to the extracellular surface of neurons by a glycosylphosphatidyl inositol (GPI) link. cpg15 was first identified from rat (Nedivi et al., 1996) and has subsequently been cloned from cat (Corriveau et al., 1999) and human (Naeve et al., 1997). In these three mammalian species, the primary amino acid sequence of CPG15 consists of 142 amino acids and shows no homology to other proteins (Nedivi et al., 1996, 1998; Naeve et al., 1997). In situ hybridization studies in mammals show that cpg15 is expressed at peak levels during development, specifically at times of afferent axonal and dendritic growth and synaptogenesis (Nedivi et al., 1996;
Naeve et al., 1997; Corriveau et al., 1999). Furthermore, cpg15 is upregulated by visual activity in adult rat visual cortex (Nedivi et al., 1996).

Viral expression of cpg15 in Xenopus optic tectal neurons promotes the elaboration of cell dendritic arbors and presynaptic axon arbors in a non-cell autonomous fashion (Nedivi et al., 1998; Cantallops et al., 2000). Electrophysiological recordings indicate that cpg15 promotes synaptic maturation by the insertion of AMPA-type glutamate receptors into synapses (Cantallops et al., 2000). The GPI link is required for these effects of CPG15, because expression of a truncated form of CPG15, lacking the GPI consensus sequence, does not promote axonal or dendritic arbor elaboration or synaptic maturation.

A thorough understanding of CPG15 function requires knowledge of the spatiotemporal expression pattern of cpg15 mRNA and protein. To address these issues, we cloned Xenopus cpg15 and mapped the spatiotemporal distribution of cpg15 mRNA and protein during nervous system development in Xenopus. cpg15 mRNA is detectable in Xenopus retina, brain, and spinal cord. Expression is detected early during nervous system development, corresponding to the time of initial morphological development and synaptogenesis. Interestingly, the protein is targeted to projection axons, where it might act as an intercellular signal to promote the development of dendritic arbors of postsynaptic neurons.

**MATERIALS AND METHODS**

All experiments were carried out in accordance with animal care protocols approved by Cold Spring Harbor Labs.

**Isolation and sequence analysis of cDNA clones**

Poly A + RNA was isolated from whole stage 47 tadpoles (Qiagen direct mRNA isolation, Qiagen, Santa Clarita, CA) and reverse transcribed into cDNA (Stratagene, La Jolla, CA). This cDNA pool was used as a template in a polymerase chain reaction (PCR) reaction, with the primers complementary to conserved mammalian cpg15 sequences (accession no. U88958) within the coding region (5’ primer: 225–247; 3’ primer: 500–524,) for 34 cycles of 95°C for 40 seconds, 40°C for 40 seconds, and 72°C for 40 seconds. A 299-bp fragment was amplified, sequenced, and cloned into pBluescript (Stratagene) and is referred to as pXcpg15-pcr. The sequence was highly homologous to cpg15 mRNA and protein during nervous system development in Xenopus. cpg15 mRNA is detectable in Xenopus retina, brain, and spinal cord. Expression is detected early during nervous system development, corresponding to the time of initial morphological development and synaptogenesis. Interestingly, the protein is targeted to projection axons, where it might act as an intercellular signal to promote the development of dendritic arbors of postsynaptic neurons.

**Northern blot**

Poly A + RNA was isolated from whole stage 47 tadpoles (Qiagen direct mRNA), and 4 μg was electrophoresed on a 1% agarose/formaldehyde gel and transferred to nylon membrane. The pXcpg15-pcr fragment used to screen the cDNA library was used as a probe prepared as described above. Hybridization was carried out under high stringency conditions (50% formamide, 50°C). Detection was carried out by autoradiography for 7 hours at ~80° (BioMax film, Kodak, Rochester, NY) in the presence of enhancer screens (Kodak).

**In situ hybridization**

For whole-mount in situ hybridizations, the pXcpg15-pcr fragment was used for in vitro synthesis of sense and antisense digoxigenin-labeled riboprobes (MAXIscript, Ambion, Austin, TX). Whole-mount in situ hybridizations were carried out as described (Harland, 1991). Briefly, embryos were hybridized overnight at 50°C in 50% formamide, and detection was carried out by using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

In situ hybridizations on frozen sections were carried out as described previously (Nedivi et al., 1996). Animals were fixed in 4% paraformaldehyde, frozen in OCT, and sectioned on a cryostat at 20 μm either as whole animals or as dissected retinas and brains. The sections were mounted on slides under RNase-free conditions, dehydrated through graded ethanol, and fixed again in 4% paraformaldehyde. αUTP 35S (Amer sham) labeled pXcpg15-pcr sense and antisense riboprobes were synthesized in vitro (MAXIscript) and hybridized on sections. 35S-labeled probes were hybridized to sections under high stringency conditions (50% formamide at 50°C) overnight, washed, and dipped in emulsion (Kodak). Slides were exposed for 3 days at 4°C and developed in developer (Kodak). Sections were counterstained with toluidine blue and viewed by darkfield and brightfield microscopy.

**Quantification of in situ hybridization**

mRNA levels were quantified as described (Corriveau et al., 1999) by using NIH Image (version 1.6). Briefly, brightfield and darkfield images were collected with a SPOT digital camera system, by using uniform settings for the entire series of images in an experiment. A single section through the dorsal optic tectum was analyzed for each of four stage 47 animals. The rostralateral and caudomedial regions of tectum were identified from and outlined on the brightfield images. The mean pixel density of the rostralateral and caudomedial regions of the tectum were measured in the darkfield images on a 0–255 scale where 255 is white. The mean pixel density of background was measured from a portion of the slide next to the tissue section and subtracted from each value separately for each section for normalization. No significant difference was found in mean pixel density of backgrounds. Statistical significance was determined by Student’s t-test. The experiment was repeated twice with animals from the same clutch, and similar results were obtained. Data are shown for one experiment only.

**Immunocytochemistry**

Tissue was fixed in 4% paraformaldehyde overnight at 4°C and cut into 20-μm cryostat sections. Sections were incubated with preimmune serum or polyclonal antiserum
to CPG15 at a 1:200 dilution in phosphate-buffered saline, pH 7.4, 5% goat serum, and 0.05% Triton X-100 as previously described (Nedivi et al., 1998). Sections were rinsed and then incubated with a fluorescein isothiocyanate (FITC)-tagged goat anti-rabbit secondary antibody (Sigma, St. Louis, MO). Immunoreactivity was imaged with a laser scanning confocal microscope (Noran Instruments, Madison, Wisconsin).

**Western blot**

Whole brains or eyes were homogenized in a glass Dounce homogenizer in buffer containing 10 mM Tris-HCl, pH 7.4, 1 mM EGTA, 0.5 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 5 μg/ml leupeptin, 20 μg/ml soybean trypsin inhibitor, and 30 mM octyl glucosid. The samples were centrifuged at 20,000 g for 2 minutes, and supernatants were collected for analysis. Samples were separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 15% acrylamide and transferred to Immobilon-P nylon membrane (Millipore, MA). Blots were blocked by using standard procedures and incubated with the same rabbit anti-CPG15 antibody (1:250) as that used for immunocytochemistry (Nedivi et al., 1998). Blots were incubated with horseradish peroxidase (HRP)-tagged goat anti-rabbit (Sigma), and detection was carried out by using standard chemiluminescence technique (ECL, Amersham). All blots were exposed for the same amount of time.

**Retinal explant cultures**

Stage 53–56 albino Xenopus laevis tadpoles were anesthetized with 0.05% MS222, and both optic nerves were crushed 4 days prior to retinal explant preparation. Tadpoles were anesthetized as before, and then both eyes were removed and kept on cold HBST buffer (5.8 mM NaCl, 0.6 mM KCl, 0.3 mM CaCl2•2H2O, 0.83 mM MgSO4•7H2O, and 5 mM HEPES, pH 7.7). The neural retina was cut diagonally into eight pieces, which were plated in pairs onto laminin-coated (5% in HBST) glass coverslips. Two to 4 hours later, culture medium was added to the explants (5 parts of L-15, 4 parts of a solution containing 95 mM NaCl, 1 mM KCl, 0.6 mM CaCl2•2H2O, 3.9 mM MgSO4•7H2O, 4 mM glutamine, and 9.4 mM HEPES, and 1 part fetal calf serum with 5 ng/ml sodium selenite, 5 μg/ml transferrin, 5 μg/ml insulin, and 2,500 units of 1% pen strep). Cultures were maintained at room temperature for 5–7 days before use. Explants were fixed and processed for immunocytochemistry as described above except following primary antibody, explants were incubated with phallloid conjugated to rhodamine (Molecular Probes, Eugene, OR) (1:800) for 1 hour.

**RESULTS**

**cpg15 is highly conserved across species**

We cloned Xenopus cpg15 from a tadpole brain cDNA library. The deduced amino acid sequence aligned with the rat CPG15 sequence is shown in Figure 1A. A probe generated from this cDNA consistently detects a single band of approximately 1.7 kb, similar to the size of mammalian cpg15 (Naeve et al., 1997) (Fig. 1B). The neural retina was cut diagonally into eight pieces, which were plated in pairs onto laminin-coated (5% in HBST) glass coverslips. Two to 4 hours later, culture medium was added to the explants (5 parts of L-15, 4 parts of a solution containing 95 mM NaCl, 1 mM KCl, 0.6 mM CaCl2•2H2O, 3.9 mM MgSO4•7H2O, 4 mM glutamine, and 9.4 mM HEPES, and 1 part fetal calf serum with 5 ng/ml sodium selenite, 5 μg/ml transferrin, 5 μg/ml insulin, and 2,500 units of 1% pen strep). Cultures were maintained at room temperature for 5–7 days before use. Explants were fixed and processed for immunocytochemistry as described above except following primary antibody, explants were incubated with phallloid conjugated to rhodamine (Molecular Probes, Eugene, OR) (1:800) for 1 hour.

**Retinal explant cultures**

Stage 53–56 albino Xenopus laevis tadpoles were anesthetized with 0.05% MS222, and both optic nerves were crushed 4 days prior to retinal explant preparation. Tadpoles were anesthetized as before, and then both eyes were removed and kept on cold HBST buffer (5.8 mM NaCl, 0.6 mM KCl, 0.3 mM CaCl2•2H2O, 0.83 mM MgSO4•7H2O, and 5 mM HEPES, pH 7.7). The neural retina was cut diagonally into eight pieces, which were plated in pairs onto laminin-coated (5% in HBST) glass coverslips. Two to 4 hours later, culture medium was added to the explants (5 parts of L-15, 4 parts of a solution containing 95 mM NaCl, 1 mM KCl, 0.6 mM CaCl2•2H2O, 3.9 mM MgSO4•7H2O, 4 mM glutamine, and 9.4 mM HEPES, and 1 part fetal calf serum with 5 ng/ml sodium selenite, 5 μg/ml transferrin, 5 μg/ml insulin, and 2,500 units of 1% pen strep). Cultures were maintained at room temperature for 5–7 days before use. Explants were fixed and processed for immunocytochemistry as described above except following primary antibody, explants were incubated with phallloid conjugated to rhodamine (Molecular Probes, Eugene, OR) (1:800) for 1 hour.

**RESULTS**

**cpg15 is highly conserved across species**

We cloned Xenopus cpg15 from a tadpole brain cDNA library. The deduced amino acid sequence aligned with the rat CPG15 sequence is shown in Figure 1A. A probe generated from this cDNA consistently detects a single band of approximately 1.7 kb, similar to the size of mammalian cpg15 (Naeve et al., 1997) (Fig. 1B). This clone contains an open reading frame encoding a predicted protein of 144 amino acids, 2 amino acids longer than mammalian CPG15. The complete open reading frame is 73% identical to that of Rattus norvegicus suggesting that it is Xenopus laevis CPG15. Xenopus CPG15 contains a conserved N-terminal secretion signal, characteristic of extracellular proteins. In addition, the six cysteine residues, which are
postulated to be critical for the correct folding of this extracellular protein, are conserved from *Xenopus* to rat and human (Naeve et al., 1997; Nedivi et al., 1998). Residues 29–33 are a consensus for S-nitrosylation (Stamler et al., 1997), and their conservation between *Xenopus* and rat suggests that this may be essential for CPG15 function. The C-terminal of *Xenopus* CPG15 is comprised of hydrophobic residues diagnostic of a GPI consensus sequence (Low, 1987). Both the N- and C-terminal signal sequences are cleaved during post-translational modification. The predicted mature protein (residues 28–115) bears a high level of homology to rat CPG15, with 73% of amino acids identical and 88% similar between the two species. No other proteins with significant similarity to CPG15 have yet been reported.

**Spatiotemporal expression of *cpg15* in the CNS**

*cpg15* is detected in stage 24 embryos in dorsal and ventral regions within the spinal cord (Fig. 2A). This is a stage at which the neurons begin to send out their axons (Hartenstein, 1993). At stage 33 (Figs. 2B,C, 3A,B), there is an increase in relative numbers of cells expressing *cpg15*, probably reflecting an increase in the number of differentiated neurons. At this stage, *cpg15* expression is prominent throughout the developing CNS and can be detected in the forebrain, midbrain, and hindbrain including the olfactory bulb and the optic tectum. *cpg15* can also be detected within the olfactory sensory neurons, the olfactory pit, auditory ganglion cells, and spinal cord (Figs. 3, 4). Little mRNA is detectable in the differentiating retina at this stage (Fig. 3A,B). *cpg15* expression is detectable within the tectum at stage 38 at the onset of retinotectal synaptogenesis (Holt and Harris, 1993) and continues to be expressed in this structure into adulthood (Figs. 3C–H, 4A–F).

*cpg15* mRNA expression is high within the telencephalon, including the olfactory bulb, parts of the thalamus, and tectum (Fig. 4). *cpg15* expression is maintained in these regions at stage 53 (Fig. 4E,F) and in postmeta-

---

**Fig. 2.** Whole-mount in situ hybridizations on albino *Xenopus* embryos. **A:** *cpg15* can be detected in dorsal spinal cord at stage 24. **B:** *cpg15* expression increases in the spinal cord and can be detected in the forebrain (FB), midbrain (MB), and hindbrain (HB) at stage 33. **C:** Higher magnification of spinal cord of stage 33 embryo. *cpg15* expression is detectable in both dorsal (arrow) and ventral cells (arrowhead) within the spinal cord. **D:** Sense probe. Rostral is to the left in all images. Scale bar = 500 μm in A,B,D; 250 μm in C.
morphic frogs (data not shown). \textit{cpg15} is expressed in the cerebellum in postmetamorphic frogs (data not shown). \textit{cpg15} appears to be expressed at higher levels in more mature neurons in rostral lateral tectum compared with recently differentiated cells in the caudomedial tectum (Fig. 4G,H). Some neurons near the border of the neuropil are very densely labeled with silver grains. We quantified the \textit{cpg15} mRNA expression in rostral lateral and caudomedial regions of the tectum (see Materials and Methods). This analysis indicates that more mature neurons in the rostral lateral optic tectum express significantly higher lev-

els of \textit{cpg15} than recently differentiated neurons in the caudomedial optic tectum (Fig. 4I). This gradient of mRNA level is consistent with the rostrocaudal gradient of neuronal development (Straznicky and Gaze, 1972).

\textit{cpg15} is not expressed by all cells. It is absent from cells in proliferative zones throughout the brain. \textit{cpg15} mRNA is also absent from the ventral telencephalon and some thalamic regions (Fig. 4C,D). Neurons in the optic tectal neuropil, which have been identified as \textgamma-aminobutyric acid (GABA)-ergic interneurons (Antal, 1991; Rybicka and Udin, 1994; Wu and Cline, unpublished data), do not express \textit{cpg15} (Fig. 4G,H). \textit{cpg15} mRNA is also absent from the nucleus isthmi (data not shown), a cholinergic midbrain nucleus involved in visual system plasticity (Udin, 1999).

In the retina, \textit{cpg15} is expressed exclusively in the RGC layer (Fig. 5). It becomes detectable around stage 38 and continues to be expressed only in the RGC into adulthood (Fig. 5E,F). No expression was detected in the proliferative zone (PZ) of the retina, as was also the case in the CNS.

\textbf{Spatiotemporal expression of CPG15 in the CNS}

Western blots indicate that CPG15 protein is detectable in brain and eye homogenates of tadpoles at stages 47, 55, and 60 and juvenile frogs (Fig. 6). CPG15 protein expression increases from stage 47 to 55 and remains high after metamorphosis.

CPG15 expression in the retina is first detectable in the optic nerve (ON) of stage 40 tadpoles (Fig. 7A). At this time we cannot detect CPG15 immunoreactivity (IR) in RGC somata. The intensity of CPG15 IR in the RGC somata and in ON increases through stage 47 (Fig. 7B,C) and is detectable in retinal axons of stage 55 tadpoles (data not shown). Tissue sections treated with preimmune serum show no label in RGC somata or axons (Fig. 7E). Although CPG15 IR can be detected in RGC somata, it is concentrated in axons within the optic nerve. Unilateral optic nerve crush at stage 55 results in a loss of CPG15 IR in the damaged nerve and tract (data not shown). CPG15 IR returns with a time-course characteristic of axon regeneration (data not shown), indicating that the IR in the ON originates from RGC axons, rather than ON glia.

To determine whether CPG15 is targeted to axon terminals, we prepared retinal explant cultures. CPG15 IR is present throughout retinal axons including the axonal growth cones of the RGC, indicating that protein is transported to these regions (Fig. 7E,F).

CPG15 IR is first detected in the brain at about stage 42/43. By stage 45 CPG15 IR is widespread in differentiated neurons throughout the CNS (Fig. 8). CPG15 antibody labels cells in a honeycomb pattern, as previously described (Nedivi et al., 1998). In dorsal sections through the brain, CPG15 IR is highest in neurons in the olfactory region of the dorsal telencephalon and in optic tectal neurons (OB and OT in Fig. 8A,B). In more ventral sections through the brain, CPG15 IR is present in neurons throughout the ventral telencephalon (Fig. 8D,E). Neurons in the tegmentum and hindbrain are immunoreactive for CPG15 (Te and HB in Fig. 8C,D).

In horizontal sections through the ventral brain, CPG15 IR is most intense in retinal axons in the optic chiasm and in axons within the hindbrain and spinal cord (Figs. 7A, 8). Retinal axons terminating in the optic tectal neuropil
Fig. 4. *In situ* hybridization of *cpg15* in brains of midstage *Xenopus* tadpoles. Darkfield images show mRNA (A,C,E,G) and brightfield images show toludine blue counterstain (B,D,F,H). 

**A,B:** Horizontal brain sections through dorsal brain of stage 47 tadpoles show *cpg15* expression in the olfactory bulb (OB), dorsal thalamus (Th), optic tectum (OT), and hindbrain (HB).

**C,D:** Sections through ventral brain show labeling in thalamus (Th), tegmentum (Te), and hindbrain.

**E,F:** Stage 53 tadpoles continue to express *cpg15* in the olfactory bulb, thalamus, and optic tectum, as well as the pallium (Pa).

**G,H:** High magnification of stage 47 optic tectum. Arrows show rostrolateral and caudomedial cells indicating the higher level of mRNA in rostrolateral tectum. Arrowheads indicate cells within the neuropil that have no detectable levels of *cpg15*. Dorsal lines in H show rostral and caudal regions of tectum used for quantification. Rostral is up in all sections.

**I:** Quantification of *cpg15* expression as mean pixel density in rostrolateral and caudomedial tectum on a 0–255 scale where 255 is white (mean ± standard error). *, \( P < 0.001 \). Scale bar = 100 \( \mu \text{m} \).
are brightly labeled (RT axons in Fig. 8A,B). Biocytin labeling of tectal neurons demonstrates tectal projections to the contralateral tegmentum and ipsilateral spinal cord (Rajan and Cline, unpublished data). Axons in these tracts are labeled by CPG15 antibody, as are tegmental axons projecting to the spinal cord (Fig. 8C,D).

CPG15 IR in the CNS remains intense through stages 49; however, by stage 55/56 CPG15 IR is decreased in the brain and was undetectable in most neurons in the CNS of postmetamorphic animals (data not shown). Labeling in the optic nerve and other axon tracts was also very low to undetectable in postmetamorphic animals (data not shown). Despite the decreased immunodetection of CPG15 in sections of older animals, Western blots indicate that CPG15 is still expressed in the CNS of these animals (Fig. 6).

In the spinal cord, we first detect CPG15 IR in commissural axons of stage 34 tadpoles (Fig. 9A). Neuronal somata in the spinal cord are not stained at this stage but become intensely immunoreactive by stage 47 (Fig. 9B). The early detection of CPG15 message and protein in spinal cord neurons suggests a potential function for CPG15 in the establishment of motor circuits.

Fig. 5. In situ hybridization of cpg15 in the retina. Darkfield images (A,C,E,G) show mRNA detection and brightfield images (B,D,F,H) show toluidine blue counterstain for the same sections. cpg15 is expressed in retinal ganglion cells (RGC) but not in the proliferative zone (PZ) of retinae from stage 38 tadpoles and at all later stages examined. In G and H, sense probe did not label any cells. Scale bar = 100 μm.

Fig. 6. Western blot analysis of Xenopus eye and brain. CPG15 antiserum detects a band of approximately 12 kD in eye and brain homogenates from stages 47, 55, and 60 and postmetamorphic (PM) Xenopus. Proteins were loaded in equal amounts (25 μg) and overloaded (75 μg) for stage 47 eye homogenate (first lane only, OL). Higher molecular weight band (~50 kD) is not detected consistently and is also present in blots incubated in preimmune serum (Nedivi et al., 1998).

DISCUSSION

Homology and conservation of CPG15

CPG15 is a 144-amino acid protein that contains an N-terminal secretion signal sequence and a C-terminal GPI consensus sequence. CPG15 is highly conserved, with 73% of amino acid identity from Xenopus to rat and 100% identity between rat and human in the mature GPI-linked protein (Naeve et al., 1997). A recently reported zebrafish EST has a predicted open reading frame encoding for a protein of 51% identity to Xenopus and 45% identity to rat CPG15 (accession no: AL877586). The high degree of con-
servation across species suggests that CPG15 performs a critical function for CNS development. It is interesting to note that no CPG15 homologue has been reported in Drosophila or any invertebrate. Drosophila do not have traditional growth factors, such as nerve growth factor (NGF) or BDNF, suggesting that the molecular mechanisms underlying developmental plasticity and synaptic plasticity differ between vertebrate and invertebrate species (Jankowsky and Patterson, 1999).

**CPG15 is targeted to axons**

Our previous studies have shown that CPG15 functions as an intercellular signaling molecule to promote both the morphological elaboration and synaptic maturation of the
retinotectal system by acting in a non-cell-autonomous fashion (Nedivi et al., 1998; Cantallops et al., 2000). Here we report that cpg15 mRNA is detectable in differentiated projection neurons within sensory systems, including the auditory, olfactory, and visual systems, and that CPG15 protein is targeted to projection axons, including the RGC axons.

The specific trafficking of CPG15 to axons is characteristic of GPI-linked proteins. The selective targeting can be most clearly seen in the retina, where cpg15 mRNA is restricted to RGCs. CPG15 IR is absent from the inner plexiform layer where RGC dendrites are located, whereas the optic nerve shows the most intense CPG15 immunoreactivity in the retina. Many developmentally regulated proteins that affect axon guidance and development are GPI-linked proteins, including semaphorins, fascilins, neural cell adhesion molecule (NCAM), and Eph ligands (Walsh and Doherty, 1997). GPI-linked proteins are concentrated in lipid rafts in the membrane where they form multimeric signaling complexes (Faivre-Sarrailh and Rougon, 1997; Tansey et al., 2000).

Under conditions of viral overexpression, CPG15 immunoactivity is detectable in tectal cell dendrites (Nedivi et al., 1998). Therefore it is possible that under these conditions, dendritically localized CPG15 signals to other tectal cells to enhance their dendritic arbor growth and synapse maturation (Nedivi et al., 1998; Cantallops et al., 2000). Alternatively, CPG15 in local tectal cell axons overexpressing CPG15 might signal to tectal cell dendrites, leading to a cascade of events culminating in enlarged tectal cell dendritic arbors and synaptic maturation. The retinal axon arbor elaboration could arise from direct signaling from CPG15-expressing tectal neurons or indirectly through a retrograde mechanism that coordinates pre- and postsynaptic morphological development, as seen in previous experiments (Zou and Cline, 1996, 1999; Wu and Cline, 1998).

Detection of CPG15 in retinal axons is consistent with a model whereby CPG15 in presynaptic axons regulates multiple aspects of circuit formation including dendritic arbor growth, presynaptic arbor elaboration, and synaptogenesis (Nedivi et al., 1998; Corriveau et al., 1999). In this model, visual activity induces cpg15 expression in RGCs. These cells in turn synthesize CPG15 protein and traffic it to axon terminals. Visual stimulation has been shown to induce cpg15 in rat visual cortical neurons (Nedivi et al., 1996). Preliminary data indicate that light stimulation induces cpg15 expression in Xenopus RGCs (Javaherian and Cline, unpublished data).

We propose that CPG15 on the surface of retinal axons signals to postsynaptic optic tectal neurons and promotes their dendritic arbor elaboration and synaptic maturation. Similarly, CPG15 expressed in the postsynaptic tectal neurons is also trafficked to their axons in the tegmentum and spinal cord, where it may affect dendritic arbor elaboration and synaptic maturation of postsynaptic partners of tectal neurons. The expression of cpg15 in axons of projection neurons, but not in local interneurons, for instance in the retina and optic tectum, supports the idea that the source of CPG15 that regulates the development of circuitry in the CNS comes from upstream sensory afferents. The expression of cpg15 in many sensory systems in tadpole brain suggests that the function of cpg15 that we propose for visual system development may be
generalizable. In particular, it is interesting to speculate that the induction of cpg15 by sensory activity may regulate the coordinated development of modality-specific circuits from the sensory periphery to motor output (Purves et al., 1996).

ACKNOWLEDGMENTS

We thank Kim Bronson and Julianya Jay for excellent technical assistance. This work was supported by grants from the NIH (to H.T.C. and E.N.), the National Down Syndrome Society (to H.T.C.), and the Ellison Medical Foundation (to E.N.).

LITERATURE CITED


