Expression of the G protein gamma T1 subunit during zebrafish development

Hui Chen,
Weis Center for Research, Geisinger Health System, Danville, PA 17822, USA

TinChung Leung,
Weis Center for Research, Geisinger Health System, Danville, PA 17822, USA

Kathryn E. Giger,
Weis Center for Research, Geisinger Health System, Danville, PA 17822, USA

Anna M. Stauffer,
Weis Center for Research, Geisinger Health System, Danville, PA 17822, USA

Jasper E. Humbert,
Weis Center for Research, Geisinger Health System, Danville, PA 17822, USA

Soniya Sinha,
Dept. of Biological and Allied Health Sciences, Bloomsburg University, Bloomsburg, PA 17815, USA

Eric J. Horstick,
Dept. of Biological and Allied Health Sciences, Bloomsburg University, Bloomsburg, PA 17815, USA

Carl A. Hansen, and
Dept. of Biological and Allied Health Sciences, Bloomsburg University, Bloomsburg, PA 17815, USA

Janet D. Robishaw*
Weis Center for Research, Geisinger Health System, Danville, PA 17822, USA

Abstract
Here, we report the identification and expression analysis of the zebrafish G protein gamma T1 subunit gene (gngT1) during development. Similar to its human and mouse homologs, we confirm zebrafish gngT1 is expressed in the developing retina, where its transcription overlaps with the photoreceptor cell-specific marker, rhodopsin (rho). Surprisingly, we also show zebrafish gngT1 is expressed in the dorsal diencephalon, where its transcription overlaps with the pineal specific markers, arylalkylamine N-acetyltransferase-2 (annat-2) and extra-ocular rhodopsin (exorh).

Analysis of the proximal promoter sequence of the zebrafish gngT1 gene identifies several conserved binding sites for the cone-rod homeobox/orthodenticle (Crx/Otx) homeodomain family of transcription factors. Using a morpholino antisense approach in zebrafish, we show that targeted knockdown of otx5 potently suppresses gngT1 expression in the pineal gland, whereas knockdown of crx markedly reduces gngT1 expression in the retina. Taken together, these data indicate that pineal- and retinal-specific expression of the gngT1 gene are controlled by different transcription factors and exogenous signals.

*Corresponding author. Tel.: +1 570 271 6659; fax: +1 570 271 6701. E-mail address: jrobishaw@geisinger.edu (J. D. Robishaw), Mail address: Weis Center for Research, 100 North Academy Ave. Danville,PA 17822, USA.

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Keywords
Zebrafish; G protein; gngT1; Signal transduction; Rhodopsin; Exo-rhodopsin; Pineal gland; Retina; Photoreceptor cells

1. Results and discussion
Heterotrimeric guanine nucleotide-binding proteins (G proteins) are an evolutionarily conserved group of proteins that transduce signals from a plethora of cell surface receptors responding to a wide variety of hormones, neurotransmitters and sensory stimuli. Structurally, the G proteins are composed of equimolar amounts of $\alpha$, $\beta$, $\gamma$ subunits. Functionally, their unique signaling roles are generally ascribed to their $\alpha$ subtypes for which 16 genes have been identified. However, this assumption is being called into question with the discovery of a similar number and heterogeneity of $\gamma$ subtypes for which 12 genes have been identified in the mouse and human genomes (Downes et al., 1999; Hurowitz et al., 2000; Robishaw and Berlot, 2004). Although the recent analyses of gene targeted mice lacking certain $\gamma$ subunit genes have begun to highlight important functional differences (Schwindinger et al., 2003; Schwindinger et al., 2004), the vast majority of the $\gamma$ subunit genes do not have assigned signaling roles more than a decade after their discovery (Robishaw and Berlot, 2004).

To better understand the functions of distinct G protein $\gamma$ subtypes, we have begun studies to characterize the $\gamma$ subunit genes expressed in zebrafish (Leung et al., 2006). In contrast to humans and mice, zebrafish offers a genetically tractable system to study the ontogeny of this important class of multi-subunit G proteins (Cheng et al., 2003). In this regard, the combined use of both in situ hybridization and reverse genetic techniques provides a powerful approach for the functional analysis of large multi-gene families expressed in this system (Leung et al., 2006). In this paper, we focus on the identification and expression analysis of the gngT1 gene in zebrafish. Since this gene has not been previously identified outside mammals (Nordström et al., 2004), information on the spatial and temporal expression of the gngT1 gene will provide a framework for future analyses of its functions in the context of the whole organism, such as zebrafish.

1.1 Zebrafish cDNA isolation and sequence analysis
The human gngT1 sequence (Scherer et al., 1996) was used to search for related sequences in the zebrafish databases. As a result of this analysis, several EST fragments were identified. Using primers corresponding to these sequences (NM199967, BI879345 and BI846907), RT-PCR was used to produce a complete zebrafish cDNA that was subjected to automated DNA sequence analysis. Translation of the open reading frame predicts a 73 amino acid polypeptide with high homology to several $\gamma$ subunit genes. Based on their primary structures, the $\gamma$ subunit genes have been divided into five subclasses, with each subclass showing less than 50% amino acid identity to members of other subclasses (Ray et al., 1995). Phylogenetic analysis (Fig. 1A) indicates that the zebrafish cDNA is most closely related to members of subclass I that exhibit a number of unique structural and biochemical properties that set them apart from the other subclasses (Balcueva et al., 2000). For instance, a characteristic feature of this subclass is the presence of a CAAX motif (where C=cysteine; A=aliphatic; and X=serine, threonine, or cysteine) that directs the addition of a farnesyl moiety to these proteins, thereby accounting for the unusual ability of this subclass to reversibly associate with the plasma membrane where signaling occurs (Ray et al., 1995; Balcueva et al., 2000). Notably, the newly identified zebrafish cDNA shares this motif (Fig. 1B).

Subclass I includes the mammalian gngT1, gngT2, and gng11 subtypes (Balcueva et al., 2000). Of these, only the gngT2 subtype has been identified outside mammals (Thisse et al.,...
Sequence comparisons indicate the newly identified zebrafish cDNA is clearly distinct from the zebrafish gngT2, with the predicted protein showing only 50.0% amino acid identity (Fig. 1B). Further comparisons reveal the zebrafish cDNA is most similar to the human gngT1 and gng11, with the predicted proteins showing 66.2 and 61.6% amino acid identity, respectively. Of the differences, seven residues are shared with the human GγT1 protein (purple residues in Fig. 1B) and three residues with the human Gγ11 protein (yellow residues). Because of its close resemblance to both the human GγT1 and Gγ11 subtypes, we cannot conclusively identify the zebrafish cDNA as one or the other subtype based on sequence analysis alone. Therefore, we examined the expression pattern of the zebrafish cDNA to provide a more definitive identification.

1.2. Tissue distribution of zebrafish mRNA
The mammalian gngT1 is exclusively expressed in the eye, where it functions in phototransduction (Kisselev and Gautam 1993; Arshavsky et al., 2002), whereas the gng11 transcript is highly expressed in the heart, lung, and skeletal muscle, where its signaling role remains elusive (Balcueva et al., 2000). To determine the tissue distribution of the newly identified zebrafish cDNA, we first performed RT-PCR analysis on a panel of adult zebrafish tissues. The zebrafish transcript shows strong expression in the eye and weak expression in the brain and testis (Fig. 2). Notably, the zebrafish transcript is not detected in the heart where high expression of the mammalian gng11 transcript is observed (Balcueva et al., 2000). Taken together, the high sequence homology and the abundant expression in the eye identify the zebrafish cDNA as the homolog of the mammalian gngT1 subunit gene. (By convention (http://zfish.uoregon.edu/index.html), zebrafish homologs of mammalian genes are given the same name and abbreviation; hence, the designation as zebrafish gngT1).

1.3. Developmental expression of zebrafish gngT1
To analyze the temporal and spatial expression pattern of zebrafish gngT1, we performed whole mount in situ hybridization on embryos at different developmental stages. At 24 hours post-fertilization (hpf), a lateral view of the embryo shows the gngT1 mRNA is expressed at a single site in the roof of the brain (Fig. 3A). At the same time point, a dorsal view of the head region shows the expression of the gngT1 gene starts from two narrow domains along the midline of the dorsal diencephalon that later merge to form the presumptive pineal gland (Fig. 3B). Between 36 and 48 hpf, the level of gngT1 expression increases but remains restricted to this region (Fig. 3A-B). Between 48 and 60 hpf, the expression of gngT1 extends to the photoreceptor layer of the eye (Fig. 3A-B).

This is the first study to show that the gngT1 transcript is expressed in a restricted region in the brain as well as the eye. To more precisely identify its localization in the brain, two-color in situ hybridization experiments were performed on 44 hpf embryos, using a digoxigenin-labeled probe corresponding to the gngT1 gene and a fluorescein-labeled probe corresponding to the aanat-2 gene. In zebrafish, the protein encoded by the aanat-2 gene regulates melatonin production in the pineal gland, and therefore, its expression serves as a useful marker for this tissue (Falcon et al., 2003). As evidenced by co-localization of these two probes, the gngT1 mRNA is expressed in the developing pineal gland in zebrafish (Fig. 3C).

1.4 Expression of gngT1 in pineal- and retinal-specific photoreceptor pathways
As the major source of circulating melatonin, the pineal gland has profound effects on the diurnal behavior and seasonal reproduction of many vertebrates (Cahill, 1996). In lower vertebrates, light acts on the pineal photoreceptor (exo-rhodopsin) to synchronize melatonin synthesis with the environmental light conditions (Cahill, 1996; Mano, et al., 1999). Comparison of the zebrafish exorh and gngT1 genes reveals that both genes share a similar temporal and spatial expression pattern in the developing pineal gland (Fig. 4A). Like
the expression of exorh is observed in the pineal gland between 24 and 72 hpf (Fig. 4A). However, unlike gngT1, the expression of exorh is not detected in the eye even by 72 hpf. Although the exo-rhodopsin receptor has been reported in the pineal gland previously (Mano, et al., 1999), the G protein subunits acting downstream of this receptor have not been identified. Two-color in situ hybridization studies on 48 hpf embryos confirms co-localization of the gngT1 and exorh transcripts in the developing pineal gland (Fig. 4B), thereby providing the first line of evidence that the products of these two genes may work together in the same signaling pathway to control melatonin production in this tissue. This is especially noteworthy since expression of gngT1 in the pineal gland has not been reported in any species (Nordström et al., 2004), although other phototransducing components have been localized there (Bönigk et al., 1996; Kasuhara et al., 2000).

In higher vertebrates, light acts on retinal photoreceptors (rhodopsin and melanopsin), which project to the suprachiasmatic nucleus to control melatonin production in the pineal gland (Pandi-Perumel et al., 2006). Comparison of zebrafish rho and gngT1 transcripts reveals that these two genes share a similar expression pattern in the retina. At 60 hpf, the expression of rho and gngT1 is readily detected in the ventral patch of the retina (Fig. 5A), the site at which the earliest differentiating neurons begin to give rise to the first photoreceptor cells (Larison and BreMiller, 1990; Robinson et al., 1995; Raymond et al., 1995; Schmitt et al., 1999). Between 72 and 84 hpf, the expression of both genes extends medially and then dorsally in a pattern that closely mimics rod cell differentiation (Hargrave and McDowell, 1992; Burrill and Easter, 1995; Raymond et al., 1995; Schmitt and Dowling, 1996) (Fig. 5A). Two-color in situ hybridization studies on 72 hpf embryos confirms that gngT1 and rho are co-expressed in the photoreceptor layer of zebrafish retina (Fig. 5B). These data support earlier studies showing the G protein γT1 subunit acts downstream of the rhodopsin receptor in the retina to produce a visual image in the cortex (Kisselev and Gautam 1993; Arshavsky et al., 2002). In addition, these results suggest that the G protein γT1 subunit might act downstream of the exo-rhodopsin receptor in the pineal gland to regulate melatonin production (Cahill, 1996; Mano, et al., 1999).

1.5. Factors controlling tissue-specific expression of zebrafish gngT1

Virtually nothing is known regarding transcriptional regulation of the G protein gngT1 gene in any species. To begin to identify the factors controlling zebrafish gngT1 expression, we identified several possible transcription factor binding sites in the 5'-flanking region of the zebrafish gngT1 gene. Of particular interest are five copies of the TAATC sequence (Fig. 6 highlighted in green). First identified in the rho and exorh genes (Chen et al., 1997; Furukawa et al., 1997; Livesey and Cepko, 2001; Asaoka et al., 2002), this consensus sequence is recognized by the Crx/Otx family of transcription factors. In mammals, Crx regulates retinal-specific expression of several phototransduction genes, including those encoding rhodopsin, arrestin, phosducin, G protein αT1 subunit, cGMP phosphodiesterase, and Crx itself (Livesey et al., 2000; Zhu and Craft, 2000; Gamse et al., 2001; Pittler et al., 2004). In zebrafish, Otx5 controls pineal-specific expression of several circadian clock genes, including those encoding aanat-2, the interphotoreceptor retinoid-binding protein, and Reverbα (Gamse et al., 2001; Appelbaum et al., 2005).

Both Crx and Otx5 bind to the same consensus sequence, suggesting that one or both of these transcription factors may be involved in regulating the expression of gngT1. To identify the relative roles of these two transcription factors, a morpholino antisense oligonucleotide (morpholino) approach was used to examine the expression of gngT1 in embryos injected with a morpholino against otx5 or crx (Gamse et al., 2001). The in situ hybridization results show that the otx5 morpholino markedly reduces gngT1 expression in the pineal gland (Fig. 7A) even though the same concentration of morpholino has little or no effect on gngT1 expression.
in the retina (Fig. 7B). Moreover, the degree of suppression is similar at 48 and 72 hpf (Fig. 7A), indicating that otx5 knockdown causes a loss rather than a delay of gngT1 expression in the pineal gland. To confirm that the otx5 morpholino does not cause a non-specific delay in the ontogeny of the pineal gland that could account for this phenotype, we monitored the development of the pineal gland in transgenic fish expressing GFP under control of the annat-2 promoter (Gothilf et. al, 2002). The finding that the control and morpholino-treated embryos express similar levels of fluorescence suggests that otx5 knockdown does not adversely affect the development of the pineal gland even though the same dose of morpholino potently inhibits the expression of gngT1 in this tissue (Compare Fig. 7A and 7C).

In contrast to the otx5 morpholino, the crx morpholino markedly inhibits gngT1 expression in the retina but has no apparent effect on its expression in the pineal gland. (Fig. 8A-B). To demonstrate that the crx morpholino does not cause a non-specific delay in the ontogeny of the retina that could account for this phenotype, we prepared transverse plastic sections through the retinas of control and morpholino-treated embryos. The results show that the crx morpholino, at the low dose used in this study, has no discernable effect on retinal differentiation even though the same dose of morpholino markedly reduces the expression of gngT1 in this tissue (Compare Fig. 8B-C).

Collectively, our results (Fig. 7-8) demonstrate that Otx5 is more important for regulating gngT1 expression in the pineal gland, whereas Crx is more important for regulating gngT1 expression in the retina. Since both transcription factors are expressed in these two tissues (Gamse et al., 2001), the basis for their differential regulation is not clear. We are currently exploring the possibility that Crx and Otx5 function in concert with other factors that may be differentially expressed between these two tissues.

1.6. Conclusions

Based on both sequence and expression analyses, we conclude that the newly identified zebrafish cDNA is orthologous to human gngT1. Although not previously identified outside mammals (Nordström et al., 2004), our most recent analysis shows that gngT1 is present in many teleost (zebrafish, Takifugu, medaka, Atlantic salmon and sockeye salmon), amphibian (Xenopus), and avian (chicken) species. Although showing 63% to 81% amino acid identity across this range of species, this level of homology for the GγT1 protein is considerably lower than that observed for other Gγ subtypes that typically show >90% amino acid identity from fish to man (Leung et al., 2006). This lower level of homology may reflect the role of gngT1 in a photoreceptor signaling pathway that has adapted to different environmental conditions.

In mammals, the gngT1 gene is positioned in tandem with the gng11 gene on human chromosome 7, where the two genes are thought to have arisen as the result of gene duplication. Since neither gene has previously been identified outside mammals (Nordström et al., 2004), it has not been possible to say when this gene duplication took place. Our analysis of the syntenic region of the zebrafish chromosome 19 coupled with an exhaustive search of the available zebrafish EST databases failed to identify the presence of the gng11 gene in zebrafish. Collectively, these results suggest that teleosts contain only the gngT1 gene and that gene duplication giving rise to the gng11 gene occurred later. Following gene duplication in mammals, the expression of gngT1 appears to have become restricted to the retina, whereas expression of gng11 appears to have emerged in the pineal gland (www.ncbi.nlm.nih.gov/projects/geo/).

Cell-specific factors controlling retinal- and pineal-specific expression of the gngT1 gene have not been determined in any species. Here, we identify Crx and Otx5 as possible transcription factors. Since both factors are co-expressed in these tissues, it is likely that Otx5 and Crx are necessary, but are not sufficient to account for the differential expression of gngT1. In this
regard, a pineal expression-promoting element (PIPE) with the sequence TGACCCCAATCT has been identified in the promoter region of exorh (Asaoka et al., 2002). We identified a sequence with partial homology to PIPE in the 5' flanking region of gngT1 (TGTCCCC; Fig. 6 highlighted in purple). In addition, we identified a second sequence with partial homology to PIPE in the first intron of gngT1 (TGACCCCCAAACC; data not shown). In future experiments, we will determine whether either of these sequence elements are critical for regulation of gngT1 expression in the pineal gland.

2. Experimental procedures

2.1 Fish Stocks

_Danio rerio_ (Florida wildtype strain; ‘That Fish Place’, Lancaster, Pennsylvania; and Longfin strain, Scientific Hatcheries, Huntington Beach, California) were maintained at 28.5°C on a 14:10 light-dark (LD) cycle. Embryos were staged according to Westerfield, 1994.

2.2 Cloning of zebrafish gngT1

RNA was isolated from 30-hpf embryos and 6-month old adults using TRIzol reagent (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized by reverse transcription reaction, and was used as a template to amplify a portion of the zebrafish gngT1 gene by polymerase chain reaction, using the primer pair: gngT1-f1 5'-GACCTCATCGTAAGCAGCAAA-3' (forward, from -22 to -2 bp) and gngT1-r1 5'-GGGATTTCCTGATCCACTTTAGC-3' (reverse, from 218 to 239 bp). A nested primer pair: gngT1-f2 5'-ATCGATATGCCGATCATAGATGTAGAAA-3' (forward, from 1 to 22 bp) and gngT1-r2 5'-CTCGAGTTAGCAAATGACACATCCACCTT-3' (reverse, from 200 to 217 bp) was used to confirm the PCR product.

2.3 RT-PCR

To determine tissue distribution, RT-PCR analysis was performed on total RNAs isolated from various adult tissues, using the ThermoScript™ RT-PCR system (Invitrogen, Carlsbad, CA) with the primer pair _gngT1-f1_ and _gngT1-r1_. The PCR amplification reaction was carried out for 30 or 34 cycles under the following conditions: 1 min for denaturation at 94°C; 45 seconds for annealing at 58°C; and 1 min for elongation at 72°C.

2.4 Whole mount in situ hybridization

The zebrafish _gngT1_ construct was generated by subcloning the PCR product described above into the Clal/Xhol sites of the pBluscript SK+ vector. The digoxigenin (Dig) RNA labeling Kit (Roche Applied Science, Indianapolis, IN) was used to generate the labeled _gngT1_ probe. A portion of the first-strand cDNA described above was used as the template to PCR amplify a portion of the zebrafish _exorh_ gene (GenBank accession number NM131212), using the primer pair: _exorh-f1_ 5'-TGCCG ATC GTCCAC TTCTC (forward, from -23 to -5) and _exorh-r15_ 5'-CTGATTGGCTGA TGGCAGC (reverse, from 1081 to 1099); or the zebrafish _rho_ gene (GenBank accession number NM131084), using the primer pair: _rho-f1_ 5'-ATGAACGGTACAGAGGGACCG-3' (forward, from 1 to 21) and _rho-r1_ 5'- CGCCGGAGACACGGGACCTGA (reverse, from 1042 to 1062). The zebrafish _exorh_ and _rho_ constructs were generated by subcloning the PCR products described above into the pCRII-TOPO dual promoter vector (Invitrogen, Carlsbad, CA). The plasmid for making the _aanat-2_ probe was provided by Dr. David C. Klein. Either the Digoxigenin (Dig) or the Fluorescein (Flu) RNA labeling Kits (Roche Applied Science, Indianapolis, IN) were then used to prepare the labeled _exorh_, _rho_, and _aanat-2_ probes.
For whole mount *in situ* hybridization, embryos were treated with 30mg/L 1-phenyl-2-thiourea (PTU) to prevent pigment formation. The staged embryos were fixed overnight with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4), and more than 30 embryos were analyzed for each observation. Whole-mount one- or two-color *in situ* hybridizations were performed essentially as described by Thisse et al, 1993, Hauptmann, 2001, and Long and Rebagliati, 2002. The immunodetection was carried out using anti-digoxigenin alkaline phosphate-conjugated Fab fragment (1:3000 dilution) or anti-fluorescein alkaline phosphate-conjugated Fab fragment (1:1000 dilution) (Roche Applied Science, Indianapolis, IN). 5-bromo-4-chloro-3-indohyl phosphate (BCIP) and 4-nitro blue tetrazolium NBT (Roche Applied Science, Indianapolis, IN) were used for single-color *in situ* hybridization. Fast Red (Sigma-Aldrich, St. Louis, MO) and NBT/BCIP were used for two-color *in situ* hybridization. The images were viewed using a MZFL3 stereomicroscope (Leica Microsystems, Wetzlar, Germany) equipped with a DEI-750D CE digital video camera (Optronics, Goleta, CA); were captured using ImagePro+ version 4.1 software (Media Cybernetics, Silver Spring, MD); and were processed using Adobe Photoshop (Adobe Systems Incorporated).

### 2.5 Antisense morpholino oligonucleotides

Two mopholino oligonucleotides targeting *otx5* (MO-otx5; 5′-CATGACTAAACTCTCTCTCTCTC-3′) and *crx* (MO-crx; 5′-ATGTAGGACATCATTCTTGGGACGG-3′) were designed according to Games et al, 2001 and were synthesized by Gene-tools (Pholomath, OR). Approximately 1nL of different concentration of MO was injected into 1-to-2-cell stage zebrafish embryos. Control zebrafish were injected with Danieau’s buffer (1×: 58mM NaCl; 0.7mM KCl; 0.4 mM MgSO$_4$; 0.6mM Ca(NO$_3$)$_2$ and 5.0 mM HEPES, pH7.6).

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### References


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Fig. 1. Phylogenetic analysis and sequence alignment of zebrafish gngT1. (A) The GenBank accession numbers for human G protein γ subunits are: GγT1 (NP_068774); GγT2 (NP_113686); Gγ2 (NP_444292); Gγ3 (NP_036334); Gγ4 (NP_004476); Gγ5 (NP_005265); Gγ7 (NP_005136); Gγ8 (NP_150283); Gγ10 (NP_001017998); Gγ11 (NP_004117); Gγ12 (NP_061329); Gγ13 (NP_057625). The GenBank accession numbers for zebrafish γ subunits are: GγT1 (NP_956261); and GγT2 (AAH59612). Phylogenetic tree prediction was performed using the TREETOP program (http://www.Genebee.msu.su/genebee.html). (B) Multiple sequence alignment was performed using the MAP program (http://searchlauncher.bcm.tmc.edu/multi-align.html). Identical residues shared by all sequences are shown in green; residues identical to human GγT1 are shown in purple; and residues identical to human Gγ11 are shown in yellow. The identity values (%) are shown at the end of the sequences. The CAAX motif is marked with a black box.
Fig. 2.
Tissue distribution of gngT1. RT-PCR analysis was performed on various tissues from 6-month old zebrafish. PCR amplification was carried out for the indicated number of cycles for gngT1 and β-actin. The latter one was used as a loading control.
Fig. 3.
Expression pattern of *gngT1* during zebrafish development. (A) Lateral view and (B) dorsal view of whole mount *in situ* hybridization of *gngT1* in the zebrafish embryos from 24 to 60 hpf. (C) Two-color *in situ* hybridization using *aanat-2* and *gngT1* probes at night (44 hpf). Fast red staining for *aanat-2* (red) and NBT/BCIP for *gngT1* (blue). Flu, fluorescein in red; Dig, digoxigenin in blue. Arrow points to the pineal and arrowheads point to the eyes.
Fig. 4.
Comparison of the gngT1 and exorh expression in the pineal gland. (A) Lateral view of the gngT1 and exorh transcripts at 24, 48 and 72 hpf. (B) Two-color in situ hybridization using exorh and gngT1 probes at 48 hpf. Fast red staining for exorh (red) and NBT/BCIP for gngT1 (blue). exorh, extra-ocular rhodopsin; Flu, fluorescein; Dig, digoxigenin.
Fig. 5. 
Comparison of the gngT1 and rho expression in the retina. (A) Lateral view of the gngT1 and rhodopsin transcripts at 60, 72 and 84 hpf. (B) Two-color in situ hybridization using gngT1 and rhodopsin probes in the retinal photoreceptor layer at 72 hpf. Fast red staining for rho (red) and NBT/BCIP for gngT1 (blue). rho, rhodopsin; Flu, fluorescein; Dig, digoxigenin. Scale bar equals 50μm.
Fig. 6.
The genomic sequence of the proximal promoter region of the zebrafish *gngT1*. The putative Crx/Otx5 binding sites are in green. The sequence motif with partial homology to PIPE is in purple and the start codon ATG is in bold. Zebrafish EST NM 199967, BI706840 were used to blast NCBI zebrafish genomic sequence. Underlined sequence is corresponding to the 5′ UTR of the zebrafish *gng T1* transcript. The GenBank accession number for this genomic sequence is BX323007.
Fig. 7.
Regulation of the zebrafish \textit{gngT1} expression by \textit{otx5} in the pineal gland. The embryos were injected with MO-\textit{otx5} and the transcripts were detected by Dig labeled \textit{gngT1} probe. (A) Dorsal view of \textit{gngT1} expression in the pineal gland. (B) Lateral view of \textit{gngT1} expression in the pineal and the retina at 72 hpf. (C) Expression of the GFP in the pineal gland at 72 hpf using the transgenic zebrafish line TG[\textit{aanat-2:GFP}].
Fig. 8. Regulation of zebrafish gngT1 expression by crx in the retina. The embryos were injected with MO-crx and the transcripts were detected by Dig labeled gngT1 probe. (A) Dorsal view of gngT1 expression in the pineal gland at 48 hpf. (B) Lateral view of gngT1 expression in the pineal gland and the retina at 72 hpf. (C) Plastic section of zebrafish eyes at 72 hpf.