Identification of a unique transcript down-regulated in the retina of rainbow trout (Oncorhynchus mykiss) at smoltification

Stephen G. Dann, W. Ted Allison, David B. Levin, Craig W. Hawryshyn*

Department of Biology University of Victoria, Victoria, Canada

Received 22 May 2003; received in revised form 25 July 2003; accepted 18 August 2003

Abstract

Developmental and physiological changes in the retina of salmonid fishes occur during smoltification, a metamorphic event associated with thyroid hormone that prepares salmon for oceanic migration. These changes include loss of ultraviolet-sensitive (UVS) cone photoreceptors, switching of visual pigments, alterations in thyroid hormone regulation, and associated changes in behavior. This model provides an opportunity to study substantial neuronal development within an established retina. Little is known, however, about how higher order neurons are altered or how retinal gene expression changes during this transition. Here, we have used differential display RT-PCR and RACE-PCR to identify a previously uncharacterized gene transcript in Oncorhynchus mykiss under developmental regulation in the retina during smoltification, rtp12.5. This unique cDNA encodes a putative protein 112 amino acids long similar to a hypothetical human open reading frame located on chromosome 14.Q24.2. Differential expression was confirmed by RNA dot blot and in situ hybridization. We also present O. mykiss sep15 cDNA sequence and describe its expression in the vertebrate retina. Considering the expression pattern within retinal tissue observed by in situ hybridization, rtp12.5 may be under TH regulation and involved in neuronal remodeling of the retina during loss of UVS cones.

Keywords: Thyroid hormone; Parr; Smolt; Selenoprotein; Salmonid fishes; Differential expression; Transcriptome analysis; Smoltification; Retina; Vision

1. Introduction

The salmonid teleost retina is an accessible model of CNS plasticity as it undergoes both morphological and physiological alterations during natural ontogeny. Loss of ultraviolet (UV) photosensitivity and UV-sensitive cones (UVS) through apoptosis in rainbow trout (Oncorhynchus mykiss) retina has been shown through histology and electrophysiology (Hawryshyn et al., 1989; Kunz et al., 1994; Deutschlander et al., 2001; Allison et al., 2003). The UVS cones in O. mykiss degenerate during smoltification, a metamorphic developmental event in the salmonid’s life history. Loss of UVS cones and UV photosensitivity can be induced in O. mykiss when treated with thyroid hormone (TH), noted for its role in development...
and metamorphosis of vertebrates (Browman and Hawryshyn, 1992, 1994a). Plate et al. (2002) illustrated the activation of TH from its inactive circulating conformation by deiodinases in the retina. TH acts through steroid nuclear receptors (THR-α and THR-β), which are transcription factors (TFs) that change gene expression levels through TH response elements within the gene’s promoter region (Wong, 2002). Similarly, exposure to endogenous retinoic acid also produces a loss of UVS cones and UV photosensitivity (Browman and Hawryshyn, 1994b). However, changes that occur in retinal gene expression have rarely been investigated, especially those associatated with the loss of UVS cones and the metamorphic transition.

Previous studies have shown that differential expression occurs in retina undergoing photoreceptor apoptosis through the study of retinitis pigmentosa and cDNA screening techniques. Differentially expressed genes in RP include clusterin, secreted frizzled-related protein and TIMP-3 (Jones et al., 1992, 1994, 2000). Clusterin likely has a cytoprotective effect in degenerating photoreceptor cells and is not causally involved in apoptosis (Jomary et al., 1999). A differential cDNA screening approach illustrated TIMP-3 expression levels increase in patients with simplex retinitis pigmentosa (Jones et al., 1994). This study was followed by one that localized TIMP-3 expression to the photoreceptor inner segments and the ganglion cell layer of retinitis pigmentosa affected retina (Jomary et al., 1995). TIMP-3 is an inhibitor of metalloproteinases responsible for remodeling of the extra cellular matrix. TIMP-3 was also implicated in the homeostasis of the retina as well as neovascularization in the diseased state (Jomary et al., 1997).

Few molecular markers are known that reflect the parr (salmonids undergoing smoltification) to smolt transition of the salmonids. Previous research has implicated genes enriched in the gill and liver including Na+/K+ ATPase, cortisol receptor, insulin-like growth factor-1 and transferrin (Sakamoto et al., 1995; Hardiman and Gannon, 1996; Mizuno et al., 2001; Singer et al., 2002). Intracerebral levels of gonadotropin releasing hormone and growth hormone releasing hormone levels have been shown to peak at smoltification in salmonids (Parhar and Iwata, 1996; Parhar et al., 1996). Identification of genes regulated in the retina at smoltification, however, is limited to the SWS1 opsin in apoptotic UVS cones. Thus, our objective was to resolve differences in retinal gene expression before and after smoltification in salmonid retina. To accomplish this we employed the differential display reverse transcription-polymerase chain reaction (DDRT-PCR) created by Liang and Pardee (1992).

The general strategy of DDRT-PCR is to amplify and isolate partial cDNA sequences from subsets of mRNAs by reverse transcription and the polymerase chain reaction with sets of arbitrary primers. DDRT-PCR is a suitable approach for the determination of differences in the transcriptome of animal, models which lack significant genetic information. DDRT-PCR was previously used to identify nocturnin, a circadian regulated gene, in Xenopus laevis retina (Green and Besharse, 1996a,b). Other studies have used DDRT-PCR to assay gene expression patterns during neuroplasticity in the central nervous system. DDRT-PCR identified tissue plasminogen activator as a factor involved in synaptic remodeling of the hypothalamus during long term depression and potentiation (Napolitano et al., 1999; Calabresi et al., 2000). Similarly, Inokuchi et al. (1996) used DDRT-PCR to identify Krox-20 as an important transcription factor in long term potentiation in the hypothalamus. We have used DDRT-PCR to successfully identify differential expression in O. mykiss retina of a novel transcript, rtp12.5, which may define a unique state of retinal development. We have compared rtp12.5’s developmentally dependent transcription to the O. mykiss homologue of human Sep15, a false positive result identified in the same DDRT-PCR experiment.

2. Methods and materials

2.1. RNA isolation

Oncorhyncus mykiss retinal tissue was dissected from six parr (whole fish average wet mass 3.42 g) and six smolt (whole fish average wet mass 68.34 g). Total RNA was isolated using a TriZol® LS Reagent (Invitrogen) extraction, a protocol modified from Chomczynski and Sacchi (1987). mRNA was subsequently isolated using the PolyAttract® (Promega) mRNA isolation system as per the manufacturers protocol. First strand cDNA synthesis was performed with SuperScript™ II reverse transcriptase (Invitrogen) using 500 ng of mRNA and 1 μl of 1 μM poly-T primer (42 °C for 60 min). The synthesized cDNA was used
as a template for subsequent DDRT-PCR experiments.

2.2. DDRT-PCR

DDRT-PCR experiments were carried out using the Delta™ Differential Display kit (Clontech) with a slightly modified protocol; PCR conditions and primers are shown in Table 1. Primers from the Delta Differential Display kit are based on commonly sequenced motifs from mammalian open reading frames although the manufacturer does not specify the species. 20 μl of DDRT-PCR reactions were combined with 95% formalin gel loading buffer, denatured at 94 °C for 5 min, and loaded on a denaturing 5% polyacrylamide/8 M urea gel in 0.5× TBE buffer. Polyacrylamide gels were run on a Bio-Rad protean II cooled slab gel system at 50 volts for 6 h. The gel was stained with 1:1000 SYBR® green I stain in 0.5× TBE and visualized on a Molecular Dynamics Storm Phosphoimaging system to identify differentially displayed bands. The gel was stained with ethidium bromide, destained and visualized with a UV transilluminator. Using the phosphoimager picture, differentially displayed bands were identified and excised from the gel with a sterile razor blade while on the transilluminator.

Denatured PCR products (i.e. single stranded DNA) were eluted in 50 μl of TE buffer and incubated for 5 min at 100 °C. The eluted DNA products were re-amplified using the same PCR conditions and primers used for the initial DDRT-PCR. Re-amplified bands were cleaned using Wizard® PCR preps (Promega) as per the manufacturer’s protocol. PCR products were T-A cloned into the vector pBluescript® (Stratagene) by pre-treating EcoRV digested vector with Taq polymerase and dTTP, and ligating DNA amplicons with T4 DNA ligase (Promega) according to the manufacturer’s protocol. Ligation reactions were used to transform electrocompetent E. coli (ElectroMAX DH10B™ Cells-Invitrogen) via electroporation. Putative positive colonies were screened by PCR using primers designed to the vector’s M13 binding sites. Five colonies containing cloned plasmid with insert of expected size were used to characterize transcripts from the isolated DDRT-PCR bands. Standard dideoxy sequencing methods on positive colony plasmids employed IRDye700 and IRDye800 labeled primers and were performed on a NEN Global IR2 DNA Sequencer System (Li-cor).

2.3. RACE-PCR for full-length sequence

5′ and 3′ RACE were used to isolate the full-length sequence of the putative differentially expressed genes. The FirstChoice® RLM Race Kit (Ambion) was used on O. mykiss retinal mRNA according to the manufacturer’s protocol, a brief description follows. Template for 5′-RACE PCR was created by first using calf intestinal phosphatase to remove free phosphates from the 5′-end of degraded or incomplete mRNAs, rRNAs or tRNAs. Full length capped mRNAs were digested by tobacco acid pyrophosphatase leaving the 5′-monophosphate necessary for adapter ligation by RNA ligase. A 45 base RNA adapter was ligated to 5′-end full length mRNA by RNA ligase. First strand cDNA synthesis was performed on the treated O. mykiss retinal mRNA by MMLV reverse transcriptase primed by random hexamers. Primers complementary to the 5′-adapter supplied with the kit were used as the forward primers in the subsequent nested PCR. 3′-RACE was accomplished by first synthesizing cDNA from O. mykiss retinal mRNA primed by a 46 bp adapter at 42 °C for 1 h with MMLV reverse transcriptase. 5′-RACE forward and 3′-RACE reverse primers were supplied with the kit. Gene specific primers were designed based on the gene fragments previously cloned and sequenced. 5′-RACE reverse primers were created from the 3′-end of the DDRT-PCR acquired fragment while 3′-RACE forward primers were created from the 5′-end of the DDRT-PCR acquired fragments. In order to confirm a single contiguous sequence for each gene PCR was performed using primers complementary to 5′- and 3′-UTR sequence. RACE-PCR and full-length PCR products were cloned and sequenced as described above in triplicate to insure sequence fidelity. All RACE-PCR and full-length PCR conditions and primers are specified in Table 1.

2.4. Confirmation of differential expression by hybridization

DDRT-PCR products were confirmed to be differentially expressed using mRNA dot blot hybridization of cDNA fragments obtained from the DDRT-PCR experiments described above (O. mykiss sep15 and rtp12.5) to parr and smolt O.
<table>
<thead>
<tr>
<th>Nested primer name</th>
<th>Annealing temperature (°C)</th>
<th>Forward primer sequence (5’ to 3’)</th>
<th>Reverse primer sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDRT-PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rtp12.5 5’ RACE 1st round</td>
<td>60</td>
<td>GTTGCTGCCCTTGACTCGAG</td>
<td>GCTGATGGCGATGAACTGACCTGAG</td>
</tr>
<tr>
<td>Rtp12.5 5’ RACE 2nd round</td>
<td>65</td>
<td>GGGTCCTTCCAGGTGTTACCG</td>
<td>CGCGATCCGAACACTGCGTTTGG</td>
</tr>
<tr>
<td>Rtp12.5 3’ RACE 1st round</td>
<td>60</td>
<td>GATGGAGAAGTGTCAAAGGGCTACA</td>
<td>GCGAGACAGAATCTACTGCTC</td>
</tr>
<tr>
<td>Rtp12.5 3’ RACE 2nd round</td>
<td>65</td>
<td>GAGACCACACAAAGTGAAAGACCC</td>
<td>CGCGATCCGAATCTACTGCTC</td>
</tr>
<tr>
<td>O. mykiss Sep 15 5’ RACE 1st round</td>
<td>60</td>
<td>TATACCTCAAATTCTCGACTG</td>
<td>GCTGATGGCGATGAACTGACCTGAG</td>
</tr>
<tr>
<td>O. mykiss Sep 15 5’ RACE 2nd round</td>
<td>65</td>
<td>CCCCAGCAGGTGACAGGAG</td>
<td>CGCGATCCGAACACTGCGTTTGG</td>
</tr>
<tr>
<td>O. mykiss Sep 15 3’ RACE 1st round</td>
<td>60</td>
<td>GTATCTCTGTTGGCTTTTCTTC</td>
<td>GCGAGACAGAATCTACTGCTC</td>
</tr>
<tr>
<td>O. mykiss Sep 15 3’ RACE 2nd round</td>
<td>65</td>
<td>GCTCCTGTGACTTGCTGTCG</td>
<td>CGCGATCCGAATCTACTGCTC</td>
</tr>
<tr>
<td>Rtp12.5 Full length</td>
<td>59</td>
<td>GATGAAAGAAAGATGGCGGTC</td>
<td>TCCATAGATGCACACTGAGG</td>
</tr>
<tr>
<td>O. mykiss Sep 15 Full length</td>
<td>59</td>
<td>TCATCATAAGGAGCTACCTG</td>
<td>CGGAAACACACACCCACCC</td>
</tr>
</tbody>
</table>

PCR was carried out using an initial denaturation of 94 °C for 4 min, followed by 28 cycles of 94 °C denaturation for 45 s, annealing temperature (see table) for 45 s, 72 °C elongation for 60 s and 1 cycle of 72 °C for 5 min.
mykiss retinal mRNA. New mRNA fractions for each hybridization experiment (six fish from each developmental stage per hybridization) were isolated using the protocol described above. Average wet mass of fish used for hybridization experiments was 3.67 g for parr (18 experimental animals) and 67.69 g for smolt (18 experimental animals). mRNA dot blot hybridization protocol was based on Christian (1992). Briefly, 500 ng O. mykiss retinal mRNA from both parr and smolt stages was vacuum transferred to Hybond™ Nylon membrane (Amersham Biosciences) and cross-linked using a UV transilluminator. Blots were hybridized with a DNA probe for rtp12.5 and O. mykiss sep15 acquired from cloning of DDRT-PCR amplicons. Probes were labeled with α-P^32 dCTP (NEN) using the Random primers DNA labeling system (Invitrogen). Hybridizations were carried out overnight at 60 °C/2× SSC/1% SDS buffer. Hybridized blots were exposed for eight hours to a Kodak phosphor screen and visual data collected on a Molecular Dynamics Storm PhosphorImager.

Similarly, the presence of rtp12.5 and O. mykiss sep 15 was examined in other tissues by northern hybridization following the protocol of Alwine et al. (1977). 500 ng of brain and liver mRNA, isolated from the same parr and smolt O. mykiss used for the dot blot hybridizations, were electrophoretically separated on a denaturing formaldehyde agarose gel. mRNA was capillary transferred to a Nylon membrane using 20× SSC overnight and hybridized overnight under the same conditions as the mRNA dot blots. Radiolabeled probes used for Northern hybridization were identical to those described above for mRNA dot blot hybridizations. All membrane hybridizations were carried out in triplicate, representative results are shown.

2.5. Localization and confirmation of differential expression by in situ hybridization

In situ hybridization was performed following the protocol of Braissant and Wahli (1998). Fresh retina were dissected from three parr (whole fish average wet mass 3.32 g) and three smolt (whole fish average wet mass 73.65 g) O. mykiss and fixed overnight at 4 °C in 4% paraformaldehyde in PBS. Retina were cryoprotected in 25% OCT in PBS overnight at 4 °C and flash frozen in liquid nitrogen for cryosectioning. Radial cryosections (12 μm) were taken from retina of parr and smolt O. mykiss on a Leica CM1850 cryostat and postfixed in 4% paraformaldehyde/PBS for 10 min at room temperature. Sections were treated twice with 0.1% active DEPC in PBS for 15 min at room temperature to inactivate endogenous RNases. Sections were washed in PBS (15 min) and equilibrated in 5× SSC (5 min, 3×). Sections were prehybridized for 4 h at 59°C covered with hybridization buffer (50% deionized formamide/5× SSC/40 μg/ml sonicated calf thymus DNA). Sections were hybridized overnight at 59 °C in the fresh hybridization buffer with the addition of fluorescein labeled riboprobes to antisense rtp12.5, antisense O. mykiss sep15 (probe length and sequence same as RNA-membrane hybridizations) sense rtp12.5 or sense O. mykiss sep15 (sense controls are of sequence complementary to antisense probes). Riboprobes were created using T3 (sense probe) and T7 (anti sense probe) RNA polymerase with incorporation of fluorescein labeled rUTP (Roche Biochemical). Hybridized sections were washed with 2× SSC/60 min/room temperature, 2× SSC/60 min/65 °C and 0.1× SSC/60 min/65 °C.

Sections were equilibrated in 100 mM Tris–HCl/150 mM NaCl, pH 7.5 and treated with RNase H (30 min at 37 °C). The sections were then incubated for 120 min at room temperature with alkaline phosphatase-coupled anti-fluorescein antibody (Roche Biochemicals) diluted 1:5000 in the same buffer, supplemented with 0.5% blocking reagent (Roche Biochemicals). Excess antibody was washed twice with fresh buffer for 15 min and then equilibrated with a 100 mM Tris–HCl/100 mM NaCl/50 mM MgCl₂ pH 9.5 buffer and developed with an NBT-BCIP (Invitrogen) colorimetric reaction in the same buffer. Color reactions were stopped by incubation of sections in 10 mM Tris/0.1 mM EDTA (pH 8.0). Photo-microscopy of in situ hybridization was performed on a Zeiss Axioskop 2 under bright field with a 40× objective. In situ hybridization experiments were repeated in triplicate to ensure expression patterns shown in the results section are accurate.

All experiments utilizing O. mykiss were in accordance with the University of Victoria Animal Care Committee under the auspices of the Canadian Council for Animal Care and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
Fig. 1. Analysis of differentially expressed transcripts isolated from retinal cDNA of parr and smolt O. mykiss. Differential Display RT-PCR performed on O. mykiss retina mRNA. Lanes 1 & 2 are amplified parr O. mykiss retina cDNA and lanes 3 and 4 are amplified smolt O. mykiss retina cDNA. Differentially expressed transcripts are identified by arrowheads. Arrowhead i denotes O. mykiss sep15 and arrowhead ii denotes rtp12.5.

3. Results

DDRT-PCR reactions from parr and smolt O. mykiss retinal cDNA were analyzed on a denaturing polyacrylamide gel (Fig. 1). Several differentially expressed transcripts were observed, two of which were isolated for further analysis due to qualitative difference in band intensity. One differentially displayed band was isolated in the juvenile retinal RNA condition (rtp12.5 at 536 bp) and another in mature retinal RNA condition (O. mykiss sep15 at 750 bp). Both amplicons were isolated and characterized by sequence analysis. Although other primer sets were used for additional DDRT-PCR experiments the results from these reactions are currently being processed and are not shown here. Subsequent RACE-PCR produced overlapping 3' and 5' sequence used to generate full-length cDNAs of O. mykiss sep15 and rtp12.5. PCR products for full-length cDNA were cloned, sequenced, and compared with the RACE results confirming their identity. The full-length clone sequences were submitted to GenBank [GenBank accession Nos. AY255832 (rtp12.5) and AY255833 (O. mykiss sep15)] and used to interrogate the translating Blastx database at NCBI (http://www.ncbi.nlm.nih.gov:80/BLAST/).

Analysis of the full length O. mykiss sep15 cDNA sequence revealed a homologue to the human selenoprotein sep15 (GenBank accession No. NP_004252 109/162 aa identities). Similar analysis of rtp12.5 sequence shows homologues for a hypothetical gene product in human (GenBank accession No. XP_040376 95/112 aa identities E-value 1e-44), mouse (GenBank accession No. XP_126985 95/116 aa identities E-value 9e-44), and Drosophila (GenBank accession No. AAF46076 37/157 aa identities E-value 2e-11). Hidden Markov Model (HMM) search through the swiss-prot database (http://www.ncbi.nlm.nih.gov/Entrez/query.fcgi?db=FASTA) reveals some homology to a protein in C. elegans (GenBank accession No. NP_499179 20/112 aa identities E-value 0.13 for Blast and 0.033 for HMM; Eddy, 1998). Alignments of the O. mykiss sep15 and rtp12.5 cDNAs to their human homologues are displayed in Fig. 2a,b.

Fig. 3a illustrates the level of expression of rtp12.5 and O. mykiss sep15 to parr and smolt O. mykiss retinal RNA. Developmental stage-specific expression was confirmed in the case of rtp12.5 and not supported in the case of O. mykiss sep15. Expression of rtp12.5 transcript is elevated in O. mykiss parr retina but absent from the retinal mRNA of smolts. Expression of O. mykiss sep15 appears the same in both parr and smolt retina, contrary to results from DDRT-PCR. The result from the DDRT-PCR in the case of O. mykiss sep15 appears to be a false positive based on the more reliable mRNA dot blot hybridizations. False positive results in DDRT-PCR are common, necessitating the use of hybridization experiments to confirm the results (Sompayrac et al., 1995).

In contrast to observations from retinal tissue, rtp12.5 and O. mykiss sep15 expression in brain
(a) *O. mykiss Sep 15* alignment to human *Sep15*

Amino acid sequence alignment of *O. mykiss sep15* to human *Sep15* (GenBank accession no. NP_004252). Bold, italicized X in each sequence (at position 94 in human) represents the selenocysteine residue encoded by the UGA codon in the mRNA sequence.

(b) *O. mykiss rtp12.5* alignment to human homologue

Amino acid sequence alignment of *O. mykiss rtp12.5* to human hypothetical protein C13ORF46 (GenBank accession no. XM_040376).

(c) Putative SECIS element in *O. mykiss Sep15* 3’ UTR

Localization of transcript expression to cellular structures within parr and smolt *O. mykiss* retina was determined by in situ hybridization (Fig. 4a–f). *Rtp12.5* appears highly expressed in the ganglion cell and inner nuclear layers of parr retina while expressed at a lower level in inner segments of some photoreceptors (Fig. 4a) but this expression is absent in the smolt retina (Fig. 4b) which appears similar to the *rtp12.5* sense probe labeling (Fig. 4c). The *O. mykiss sep15* transcripts showed expression in the ganglion cell layer, the inner...
Fig. 3. *O. mykiss* retinal mRNA hybridizations to *rtp12.5* and *O. mykiss sep15* probes. (a) Dot blot hybridization of *O. mykiss sep15* (top) and *rtp12.5* (bottom) to parr and smolt *O. mykiss* retina mRNA. *O. mykiss sep15* hybridization shows constitutive expression between the parr and smolt stages. *Rtp12.5* hybridizes a transcript showing high expression in the parr *O. mykiss* retina, and low in the smolt stage. (b) Multiple tissue Northern hybridization of *O. mykiss sep15*. Lanes 1 and 2 are parr and smolt *O. mykiss* liver mRNA, respectively. Lanes 3 and 4 are parr and smolt *O. mykiss* brain mRNA, respectively. (c) Multiple tissue Northern hybridization of *rtp12.5*. Lanes as per B.

nuclear layer and the outer nuclear layer of both parr and smolt retina (Fig. 4d,e). Background staining of retinal tissue was assessed by using hybridization of the sense riboprobe to parr *O. mykiss* retina (Fig. 4c,f). Differential expression of the *rtp12.5* transcript in the retina has been described through DDRT-PCR analysis, mRNA dot blot hybridization and in situ hybridization. Although the *O. mykiss sep15* appears differentially displayed in the original PCR experiment, we could not confirm differential expression by RNA hybridization experiments. Although the *O. mykiss sep15* levels do not appear to change based on the in situ hybridization results, it may be hard to rule out slight changes using the techniques presented here due to signal sensitivity. The apparent lack of differential expression of *O. mykiss sep15*, however, makes it a suitable internal control for the stage specific *rtp12.5* transcript.

4. Discussion

We have isolated two genes previously uncharacterized in teleosts, or in the retina of any species, from the transcriptome of *O. mykiss* retina. Previous research has characterized the *Sep15* gene from humans and rodents in prostate, liver, kidney, testis, and brain (Gladyshev et al., 1998). *Sep15* has been implicated in cancer etiology and is associated with UDP-glucose:glycoprotein glucosyltransferase that is responsible for protein folding in the endoplasmic reticulum (Kumaraswamy et al., 2000; Korotkov et al., 2001; Kumaraswamy et al., 2002). This is the first description of a *Sep15* transcript localized in a vertebrate retina and the first description of *Sep15* in a non-mammalian organism. We could not confirm differential expression of *O. mykiss Sep15* in the retina of rainbow trout. This was likely due to a false positive result, common in DDRT-PCR screens. Considering the importance of selenoproteins in strong redox reactions of cellular metabolism and the proposed role of *Sep15* in protein folding it is not surprising to see a lack of differential expression between parr and smolt trout.

A selenocysteine insertion sequence (SECIS) element in the 3′-UTR identified in the human *Sep15* also appears to be present in the *O. mykiss* mRNA sequence as predicted by RNA structure v3.7 (Mathews et al., 1999, Fig. 2c). The SECIS in human selenoproteins produces a stem-loop structure responsible for signaling selenocysteine
tRNA insertion at the UGA codon as opposed to the truncation of the mature peptide at what is normally a stop codon (Shen et al., 1993; Walczak et al., 1997). We predict that the SECIS like element in the 3'-UTR of the *O. mykiss* sequence also directs this modification during translation. If so, the 1043bp cDNA will encode a mature protein 157aa with a selenoysteine residue located at position 84. Follow up studies using ^75^Se tagged protein fractions that show a 15 kDa Se-labeled protein would confirm this prediction.

*Rtp12.5* is differentially expressed in the *O. mykiss* retina between parr and smolt developmen-
tal stages (smoltification). The novel cDNA sequence of *rtp12.5* in *O. mykiss* is 911bp and encodes a putative protein of 112 aa in length. The predicted protein mass of *rtp12.5* is approximately 12.5 kDa with a pI of 4.80 (rainbow trout protein 12.5 kDa). PHD prediction was carried out (http://www.embl-heidelberg.de/predictprotein/ predictprotein.html) to determine likely tertiary structure of *rtp12.5* (Rost and Sander, 1993, 1994). This analysis reveals a tertiary structure of almost entirely alpha helix interrupted only by loops located at proline residues. Three sites in the protein sequence reveal two types of possible post-

Fig. 4. In situ hybridization of *O. mykiss* *sep15* and *rtp12.5* to parr and smolt *O. mykiss* retinal tissue cryosections. (a) Antisense RNA probe in situ hybridization of *O. mykiss* *rtp12.5* to parr retinal tissue. (b) Antisense RNA probe in situ hybridization of *O. mykiss* *rtp12.5* to smolt retinal tissue. (c) Sense in situ hybridization of sense *O. mykiss* *rtp12.5* probe to parr retinal tissue. (d) Antisense RNA probe in situ hybridization of *O. mykiss* *sep15* to parr retinal tissue. (e) Antisense RNA probe in situ hybridization of *O. mykiss* *sep15* to smolt retinal tissue. (f) Sense RNA probe in situ hybridization of sense *O. mykiss* *sep15* to parr retinal tissue. Hybridization signal is the black precipitate (developed by an NBT/BCIP alkaline phosphatase mediated reaction) in the micrographs as indicated by arrows.
translation modification. A serine residue, at position 41, is a candidate for protein kinase C phosphorylation. Glycine residues at position 69 and 91 are probable sites for acetylation by covalent amide linkage of myristate via N-myristoyl transferase.

We have used the genome database users guide to navigate the human genome surrounding this previously uncharacterized gene product (Wolfsberg et al., 2002). The human genome map reveals a hypothetical gene (GenBank accession No. XM_040376 E-value 1e-44) on chromosome 14 at site 14.024 with the official name Chromosome 14 open reading frame 46 (symbol C14orf46). Based on amino acid sequence and HMM search, this gene may be similar to a nuclear protein identified by in vivo RNAi screening of expression in C. elegans (GenBank accession No. NP_499179 E-value 0.13 in Blast, 0.033 in HMM searches). Knockdown of the expression of this gene resulted in deficiencies in egg laying abilities, abnormal oogenesis and reduced fertility in C. elegans (Maeda et al., 2001). Although the HMM search reveals some homology with the C. elegans gene it is likely only an indication of rtp12.5's gene family and not a functional description. There is no identified homology of rtp12.5 to any zebrafish mRNA or genomic contig assembled in the NCBI zebrafish database as additions to this genome project are ongoing.

The expression level of rtp12.5 transcripts may be correlated to the loss of UV sensitivity and UVS cones based on the stage-specific disappearance of rtp12.5 from the retinal mRNA (Deutschlander et al., 2001, Allison et al., 2003). Based on the higher expression levels of rtp12.5 transcript in the inner nuclear and ganglion cell layers of the O. mykiss retina, as opposed to the lower levels observed in the photoreceptor layer, it is possible that rtp12.5 is involved in neural remodeling during UVS photoreceptor apoptosis. If this is the case it implies that at least a subset of the higher-order retinal neurons are changing their gene expression depending on the compliment of photoreceptor inputs they are processing. Such inputs might include chromatic information (especially in the UV range) and/or polarization sensitivity, both of which are known to be lost after smolification (Hawryshyn et al., 1990; Hawryshyn, 2000; Allison et al., 2003). It is also likely, however, that the rtp12.5 gene is a housekeeping gene involved in protein synthesis and/or metabolism based on the high expression levels in other tissues.

We have shown that gene expression within the retina of the O. mykiss changes depending on the stage of the organism’s life history. Using differential display RT-PCR, we have isolated two genes putatively differentially expressed between parr and smolt retina. It is likely that the use of more primer sets to perform differential display experiments will reveal more genes whose expression is varied over the parr–smolt transition. A homologue of the human Sep15 appears to be constitutively expressed through dot blot and in situ hybridization. Although a smaller change in O. mykiss sep15 expression may not be detectable with the techniques presented here future quantitative RT-PCR and gene-chip screening experiments may reveal some differential expression in the salmonid retina. Rtp12.5, a gene previously characterized only as a hypothetical genome annotation in human, mouse, and Drosophila, appears to be tightly regulated in the retina of O. mykiss in a developmental stage specific manner. A putative nuclear protein involved in oogenesis from C. elegans also shows some homology to rtp12.5 although this relationship may only reflect the same gene family. Retinal development in the salmonids as well as amphibians is TH dependent and result in extensive changes in gene expression. It will be interesting to examine whether rtp12.5 is involved in UVS cone loss, neuronal remodeling or is TH responsive. Further study will attempt to identify a protein product of rtp12.5 and determine its function in the parr retina. Knockdown studies of rtp12.5 in the retina of O. mykiss via siRNA may also provide functional information regarding its differential expression in the retina.

Acknowledgments

The authors would like to thank the sources of funding that made this research possible. In particular, the Natural Sciences and Engineering Research Council of Canada (Equipment and Operating grants to CWH; PGS-B Scholarship to SGD) and the Alzheimer Society of Canada/BC and CIHR Institute of Aging (Doctoral fellowship to WTA). The authors would like to thank Kathy and Nik Veldhoen for comments on the manuscript prior to submission as well as the anonymous reviewers that took part in the peer review process. We would also like to thank the Vancouver Island
Trout Hatchery for supplying experimental animals.

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