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A comprehensive survey of the genes involved in maturation and development of the rainbow trout ovary

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ABSTRACT

The development and maturation of the ovary requires precisely coordinated expression of specific gene-classes to produce viable oocytes. We undertook identification of some of the genes involved in these processes by creating ovary-specific cDNA libraries by suppression subtractive hybridization and by microarray-based analyses. We present 5778 tissue- and sex-specific genes from subtracted ovary and testis libraries, many of which remain unidentified. A microarray containing 3557 salmonid cDNAs was used to compare the transcriptomes of precocious ovary at three different stages during second year with a reference (normal ovary) transcriptome. On average, about 240 genes were developmentally regulated during the study period from June to October. Classes of genes maintaining relatively steady-state levels of expression, such as those controlling tissue remodeling, immunoregulation, cell-cycle progression, apoptosis and growth were also identified. Concurrent expression of various cell division and ubiquitin-mediated proteolysis regulators revealed the utility of microarray analysis to monitor important maturation events. We also report unequivocal evidence for expression of the transcripts that encode the

common glycoprotein-α (Cgα), LHβ, FSHβ and TSHβ subunits, and retinol-binding protein in both the ovary and testis of trout.

INTRODUCTION

Development of reproductive tissue is a dynamic process involving coordinated interactions between regulators that assemble or edit the cellular constituents that support the developing gametes [1-3]. Endocrine and locally expressed steroids and hormones induce growth, differentiation and maturation of the follicular cells [4-6]. Both the assembling support structures and the maturing follicles undergo cellular remodeling and organization throughout development.

Bidirectional communication occurs between both the oocytes and somatic follicular cells. Oocyte-secreted factors regulate granulosa cell differentiation, proliferation and function, whereas granulosa cell paracrine activities ensure the growth and development of the oocyte [5, 6]. Changes in expression of the components that comprise the connective tissue matrix also participate in follicular maturation and function [3, 7]. There also is some evidence that immune cells interact with and coordinate the function of the somatic cells associated with germ cells [8, 9]. These complex processes must provide the precise regulatory and physiological milieu for production of functional gametes.

One interesting phenomenon found in a small percentage of juvenile salmon is that they are ready to undergo spawning at least a year ahead of their siblings. These precocious males and females undergo dramatic increases in growth and development of their testes and ovaries in comparison to their normal (“less mature”) cohorts. This provides an opportunity to compare and characterize the genes expressed in immature, normal and precocious reproductive tissues of the same age.

To understand what genes are involved in these dynamic developmental processes, we undertook the following study. First, to identify some of the genes expressed differentially in normal and precocious ovary, we constructed subtracted cDNA libraries using immature tissue as the reference cDNA population. Second, we used 3557-gene salmonid cDNA microarrays to

profile gene expression at three stages of precocious ovary development (June, August and October precocious) relative to reference (June normal) ovary. We also followed the expression of several genes heretofore considered to be absent from or only weakly expressed during ovarian development.

MATERIALS and METHODS

Animals

Each gonadal tissue used in this study was obtained from 1.5 to 1.8 year old male or female rainbow trout (*O. mykiss*) raised in an open lake fed by a natural stream in Sooke, British Columbia (Mountain Trout Sales). Most trout ovulate and spawn for the first time at 3 yr and then continue to spawn annually. However, 10 to 20% of trout mature precociously, beginning at about 1.5 yr of age, and may spawn at 2 yr, one year ahead of their normal ("less mature") cohorts. Precocious maturation is a normal reproductive state in which offspring can be produced. Fish were judged to be precociously mature on the basis of the weight of the gonads and of other defining characteristics such as visible eggs, orange coloration and larger size of ovaries in comparison to their normal cohorts. Gonadal tissue was considered immature if it was sexually indeterminant by visual inspection.

Tissue and RNA extraction

Fish were exsanguinated for several minutes. The tissues were removed and flash frozen in liquid nitrogen and stored at -80˚C until RNA extraction. Flash frozen tissues were ground using baked (220°C, 5h) mortars and pestles under liquid N_2, then total RNA was extracted in TRIzol reagent (Invitrogen, Carlsbad, CA) and poly(A)+ RNA was purified using MicroPoly(A)Pure kits (Ambion, Austin, TX).

Subtractive Hybridization

Total RNAs extracted from April precocious ovaries and testes, normal ovaries and testes and immature tissues were obtained from several animals (except precocious tissues) due to quantity differences based on the different maturation states of each tissue. Poly(A)+ RNAs were

converted into cDNAs and reference (driver) and experimental (tester) cDNAs were subjected to suppression subtractive hybridization (SSH) using the PCR-Select cDNA Subtraction kit according to the manufacturer's instructions (Clontech, Palo Alto, CA). A SSH library is enriched for cDNAs that are more abundant in the tester than in the driver. In subtractive hybridizations, precocious ovary and testis and normal ovary and testis cDNAs were used individually as tester against driver naïve cDNA. In addition, reciprocal SSH libraries were generated from normal ovary and normal testis cDNAs.

Products from secondary PCRs amplified using the Advantage cDNA PCR kit (Clontech) were size-fractionated on a 1.0% agarose gel. Insert sizes of cDNA libraries were determined by visual comparison of clone restriction fragments with the DNA size markers *Hind*III and 1 kb ladder. High (500-1500 bp) and low (200-500 bp) molecular weight (MW) cDNAs were subcloned into pCR4-TOPO vector and transformed using Top10 electrocompetent cells (Invitrogen). 7936 randomly selected clones from the 12 sublibraries (high and low MW libraries) were extracted and sequenced by BigDye Terminator (ABI, Foster City, CA) cycle sequencing on an ABI 3700 sequencer using conventional procedures and M13 forward and M13 reverse primers. Base-calling from chromatogram traces was performed using PHRED [10, 11]. Vector, poly-A tails, and low quality regions were trimmed from each sequence; sequences that had less than 100 good quality bases after trimming were discarded.

Microarray Fabrication and Quality Control

Library construction, gene selection, microarray fabrication and quality control of the array used in this study have been described in detail [12]. Briefly, 3557 cDNA clones from 18 high complexity salmonid cDNA libraries/library groups were selected and printed as double, side-by-side spots on ArrayIt Superamine slides (Telechem Int., Sunnyvale, CA) with the Biorobotics Microgrid II microarray printer (Apogent Discoveries, Hudson, NH). Microspot 10K quill pins (Biorobotics, Cambridge, UK) in a 48 pin tool were used to deposit approximately 0.5 nl (0.2 ng cDNA) per spot onto the slide. The resulting microarrays have a 4-by-12 subgrid layout

with 132 spots per subgrid, each spot having approximate diameter and pitch of 100 μm and 250 μm respectively. The slides were crosslinked in a UV Stratalinker 2400 (Stratagene, La Jolla, CA) at 120 mJ. Spot morphology was assessed by visual inspection or by SYBR Green 1 (Molecular Probes, Eugene, OR) staining.

Microarray Hybridization and Analysis

This microarray experiment was designed to comply with MIAME guidelines [13]. To minimize technical variability, all targets were synthesized in one round. Total RNA was extracted (TRIzol, Invitrogen) from flash-frozen precocious (June, August and October) and normal (June) ovarian tissues collected from rainbow trout in second year. Extracted total RNAs were cleaned using MEGAclear (Ambion) and then quantified and quality-checked by spectrophotometer and agarose gel, respectively. The microarray experiment used June normal ovary as reference and included 3 replicates (two identical and one dye-flip) for comparison of each precocious ovary stage with the reference sample. Nine microarrays were used in total: 3 June precocious vs. June normal ovary, 3 August precocious vs. June normal ovary and 3 October precocious vs. June normal ovary.

Hybridizations were performed using the Genisphere Array50 version 2 kit and instructions (Genisphere, Hatfield, PA). Briefly, 11 μg total RNA were reverse transcribed using oligo d(T) primers with unique 5'-sequence overhangs for the cyanine fluor Cy5 or Cy3 labeling reactions. Microarrays were prepared for hybridization by washing 2 X 5 min in 0.1% SDS, washing 5 X 1 min in MilliQ H_2O, immersing 3 min in 95˚C MilliQ H_2O, and drying by centrifugation (5 min 2000 rpm in 50 ml conical tube). The cDNA was hybridized to the salmon cDNA microarray in a formamide-based buffer (25% formamide, 4X SSC, 0.5% SDS, 2X Denhardt's solution) 16 h at 48˚C. The arrays were washed 1 X 10 min in 48˚C (2X SSC, 0.1% SDS), 2 X 5 min in (2X SSC, 0.1% SDS) at room temperature (RT), 2 X 5 min in 1X SSC at RT, and 2 X 5 min in 0.1X SSC at RT, and dried by centrifugation. The Cy5 and Cy3 3-dimensional

fluorescent molecules (3DNA capture reagent, Genisphere) were hybridized to the bound cDNA on the microarray with 3DNA capture reagents bound to their complementary cDNA capture sequences on the oligo d(T) primers. The second hybridization was done 3 h at 48˚C, and washed and dried as described above.

Fluorescent images of hybridized arrays were acquired immediately at 10 mm resolution using ScanArray Express (PerkinElmer, Wellesley, MA). The Cy3 and Cy5 cyanine fluors were excited at 543 nm and 633 nm, respectively, and the same laser power (90%) and photomultiplier tube (PMT) settings were used for all slides in the study (Cy3: PMT 73; Cy5: PMT 67). Fluorescent intensity data was extracted from TIFF images using Imagene 5.5 software (Biodiscovery, El Segundo, CA). Quality statistics were compiled in Excel from raw Imagene fluorescence intensity report files. Elements were sorted (7356 salmonid spots representing 3557 different cDNAs, 20 Arabidopsis spots representing 5 different cDNAs, and 1356 other control spots) and median signal values and mean numbers of salmonid elements passing threshold were determined for Cy3 and Cy5 data separately. Data analyses (background correction, Lowess normalization, and fold change gene list formation) were performed in GeneSpring 6.1 (Silicon Genetics, Redwood City, CA). For a microarray feature to be included in an informative transcript list, its background-corrected Lowess normalized (BCLN) Cy5/Cy3 ratio had to be either >2.0 (Table 1) or < 0.5 (Table 2) in all three pertinent slides. For Tables 1 and 2, fold change values (ratios) were calculated with the dominant channel (the higher expression sample, i.e. precocious for Table 1 and normal for Table 2) in the numerator. For Tables 3 to 6, all fold change values were calculated with BCLN precocious sample values in the numerator. For each transcript of interest, fold change values were entered into an Excel spreadsheet. Mean, standard deviation and standard error calculations were made across replicate microarrays in Excel. All scanned microarray TIFF images, extracted ImaGene grid files, the gene identification file, ImaGene quantified data files and quality statistics are available on-line as supplemental data (http://web.uvic.ca/cbr/grasp).

PCR

The primers used to amplify Cgα, LHβ, FSHβ, TSHβ, RBP and ubiquitin (control) were designed specifically against the sequences provided for each rainbow trout gene obtained from http:// www.ncbi.nlm.nih.gov with the following accession numbers: AB050834 for Cgα; AB050836 for LHβ; AB050835 for FSHβ; D14692 for TSHβ; AF257326 for RBP; AB036060 for ubiquitin. For each gene, sequences of the forward and reverse primers used in each respective PCR, are as follows: Cgα, 5'-CAACATCATGCAGTGTACAGG-3' and 5'-ATCAGTATTCAATTCATACAG-3'; LHβ, 5'-GATGTTAGGTCTTCATGTAGG-3' and 5'-CAAGTACATTCACATACAACC-3'; FSHβ, 5'-TGCCGACTAAACAACATGACC-3' and 5'-TGCAATAGCACATCAACAATG-3'; TSHβ, 5'-CTGCTCTTCAGCCAAGCTGTG-3' and 5'-AACACACGAGTACGACAATGC-3'; RBP, 5'-CAATGTCGTCGCTCAGTTCT-3' and 5'-TCAACTGCTTTCACAGAAAC-3'; ubiquitin, 5'-ATGTCAAGGCCAAGATCCAG-3' and 5'-TAATGCCTCCACGAAGACG-3'.

cDNAs were synthesized in 25-µL reactions that contained 200 ng of poly(A)+ RNA or 1.0 µg total RNA using Omniscript RT by manufacturers instructions (Qiagen, Mississauga, ON). The reactions were incubated at 37°C for 90 min and the transcriptase heat-inactivated at 70°C for 30 min. Approximately 200 ng of cDNA was used in each 25-µL PCR reaction containing 1.25 U Taq polymerase , 1 X Taq buffer, 1.25 mM $MgCl_2$, 10 mM dNTPs (Invitrogen) and 15 pmol of each gene-specific 5' and 3' primer. Each PCR was carried out under the following cycling parameters: 94°C for 2 min, then 40 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min using a Perkin Elmer 9600. The PCR products were separated by electrophoresis on 1.0% agarose gels and photos stored using an Eagle Eye II still video system (Stratagene). Representative products were isolated and cloned into pCR4-TOPO vector and sequenced to confirm gene identities.

RESULTS

SSH Libraries

To identify potential gonad-specific and sex-specific genes, we used suppression subtractive hybridization (SSH) as a technique to create 12 sublibraries. From these libraries a total of 7936 clones were M13 forward-sequenced and quality checked. Of these clones, 5778 cDNAs passed quality filtering processing. Access to data related to each of these gene fragments can be found at http://web.uvic.ca/cbr/grasp. For the two ovarian tissue classes examined (precocious and normal) there are 1722 different cDNAs (see libraries rtah, rtal, rtch, rtcl); for the testicular counterparts there are 2318 different genes reported (see rtbh, rtbl, rtdh, rtdl). We also report 639 and 1099 genes that are differentially expressed between normal ovary and testis (see rteh, rtel) and between normal testis and ovary (see rtfh, rtfl), respectively.

Microarray analysis

Differential gene expression in the three developmental stages of precocious ovary (June, August and October) relative to June normal ovary was determined using a microarray presenting 3557 different cDNAs selected from 18 high-complexity salmonid cDNA libraries [12]. Genes from libraries of ovarian or testicular origin have 281 representatives on this array. The majority of cDNAs selected for the chip came from a normalized mixed tissue library (*S. salar* spleen, kidney and brain).

Data analysis executed in GeneSpring 6.1 permitted the passage of 2852 genes. There were 263, 164 and 304 genes greater than two-fold upregulated and 220, 146 and 348 genes greater than two-fold downregulated in June, August and October precocious ovary (relative to June normal ovary), respectively (Tables 1 and 2). Only those cDNAs which were above or below the 2-fold lines in two or more stages of analysis were included in these Tables. In cases where there were multiple hits for the same gene name only the best candidate was included in Table 1 or 2. The presence of multiple entries of some genes served to provide an internal validation of our microarray results. For example, there were 5 prostaglandine D synthase, 2 fatty acid binding protein H-FABP and 2 simple type II keratin K8a microarray elements in the

original “genes upregulated in precocious ovary relative to normal ovary” gene list contributing to Table 1. Also, those genes possibly of little interest to the focus of this experiment (such as ribosomal RNAs, general housekeeping genes) were not included in Tables 1 and 2.

The most highly upregulated transcript in this study was the complement receptor type 2 (CR2) (av. 27.53 fold; SEM 5.79) (Table 1). Several other immunoregulatory genes (such as several histocompatibility antigens, complement components and immunoglobulins) were also found to be upregulated in precocious relative to normal ovary. We present data for 31 other potential immunoregulatory genes that were not differentially expressed between precocious and normal ovary (Table 3).

We also observed the steady-state expression of a number of ubiquitin-proteosome components, cell division regulators and apoptotic factors (Table 4). Expression of some of these genes could point to both proteolytic and nonproteolytic activities, some of which might be key to meiotic and/or mitotic control mechanisms. Coexpression of at least 5 of these genes (Table 4; in bold) defines an important period in which follicular maturation undergoes a steroidogenic shift. Furthermore, the products of genes such as elastase IIIA, cathepsins and nidogen (Table 1) and alpha-2-macroglobulins, alveolin and TIMP2 (Table 2) have been implicated in cellular assembly and editing. Six more genes with similar functions that were expressed at steady-state levels are included in Table 5.

Only cDNAs having significant ($E < 10^{-5}$) BLASTX hits against the current GenBank databases are described for genes in Tables 1 and 2 (> 2-fold up- or downregulated in precocious ovary relative to normal ovary) or Tables 3 to 6 (similar expression levels in precocious and normal ovary). For each table, a GENBANK accession number is provided for each expressed sequence tag (EST) corresponding to each microarray element. Not available (n/a) is indicated where the EST has not yet been submitted. To identify potentially informative genes, the degree of similarity (length and percent identity over aligned region) between salmonid microarray element EST translations and their most significant (most negative E-value) BLASTX hits are

presented (Tables 1 to 6). If a salmonid EST has no significant BLASTX hit, then the most significant BLASTN hit (n) is shown.

Changes in transcription of informative genes are provided for each stage (June, August and October) of precocious ovary development relative to normal June ovary and shown as mean fold change (MFC) with standard error mean (SEM) (Tables 1 to 6). The MFC values presented in each Table are organized in descending order by June precocious ovary MFC.

Identification and confirmation of uniquely-expressed genes

Microarray analysis revealed the steady-state expression of various important growth factors, cytokines and hormones (Table 6). One unexpected finding was the hybridizations to the array of transcripts that encode the pituitary glycoprotein hormone subunits shown in Table 6 (in bold). To confirm these results and to investigate how broadly some of these transcripts might be expressed, we used PCRs to amplify cDNA taken from tissues at various development states (Fig. 1). The expression of RBP was also followed because the presence of this gene had not previously been unequivocally demonstrated in either ovary or testis of fish [14, 15]. PCR products of the following sizes were generated using specific primer sets for each gene: Cgα, 462 bp; LHβ, 587 bp; FSHβ, 414 bp; TSHβ, 549 bp; RBP, 417 bp and ubiquitin, 158 bp.

DISCUSSION

A coordinated interplay of signals are required to regulate the proliferation, differentiation, adhesion and migration of specific cell types for development and organization of the ovarian structural tissues. This dynamic cellular matrix leads to the formation of the nerves, vasculature and lymphatics within the stroma of the developing ovaries. The developing follicle is derived from germinal epithelium, while the outer thecal layers are stromal derivatives [16]. The outer thecal cell layers of the growing follicles are separated from the granulosa cell layers by a distinct basement membrane containing fibroblasts, collagen fibers and capillaries [16, 17]. Many

different types of collagens, globins, keratins and lectins required for the formation of the supporting connective tissue and developing follicles were developmentally regulated (Tables 1 and 2). Concomitant with these activities we show increased expression of transcripts that encode various elastases, metalloproteinases, cathepsins and serine and cysteine proteases which participate in remodeling of the extracellular matrix (ECM) and basement membrane structures (Tables 1, 2, 5).

Regulation of ovarian cellular organization and modeling

A fine balance of the spatiotemporal expression of some of these messages must occur during organization and modeling of the supporting tissues and during oocyte development. For example, differences in the timing and expression levels of cathepsins K, L and S are shown in Table 1. These cysteine proteinases have been demonstrated to possess collagenolytic activities that degrades ECM and basement membranes [2]. Cathepsin L, together with cathepsin D (expressed between 2-fold lines), have activities that have also been associated with yolk processing during vitellogenesis in rainbow trout [18].

A trout ovulatory protein-2 (TOP-2) with potential anti-elastase or anti-cathepsin activity [19] is also expressed at dramatically increasing levels during the period from June to October. Interestingly, the strong expression of the TOP-2 transcript coincides with increased expression of a pancreatic elastase transcript (Table 1). The marked increase in expression of a serine protease through the period of this study also correlates with Northern blot and densitometric analysis of this transcript in preovulatory and ovulatory brook trout ovarian tissue [20].

The expression of transcripts that inhibit proteolysis, such as tissue inhibitor of metalloproteinase 2 (TIMP2) and alpha-2-macroglobulins 1 and 3, are downregulated in these tissues (Table 2). Concurrent with the declining expression of TIMP2, we observe decreased expression of alveolin (a metalloproteinase) and steady-state expressions of various elastases (Tables 2 and 5).

Interestingly, a variety of matrix metalloproteinases, elastases and inhibitors were isolated from normal ovary-specific subtracted libraries using normal testis cDNAs as the reference population (see http://web.uvic.ca/cbr/grasp). These activities were not identified in the testis-specific subtracted libraries. Although less than 2000 genes in this category were sampled, this observation could point to differences in the timing of transcription of these morphogenic factors between normal ovary and testis of the same age.

Presence of immunoregulators in the developing ovary

Upregulation of complement receptor type 2 (CR2) and various complement factors and immunoglobins were detected in this study (Table 1). Many immune factors potentially involved in the development of the ovary that were expressed at steady-state levels are also shown in Table 3. The complement system is activated primarily by two pathways, the classical and alternative pathways. The classical pathway is triggered by antigen-antibody complexes and the alternative pathway is initiated on cell surfaces in the absence of antibodies. The regulated and steady-state expressions of CR2, complement C1q and various downstream complement components and immunoglobins (Tables 1 and 3) indicates potential complement activation by both pathways. Both of these arms of the complement cascade could be initiated for tagging and removal of apoptotic cells and cellular debris from tissues undergoing considerable growth and remodeling. Some members of the complement cascade may also be involved in modulating changes in the ECM through proteolytic activities that modify the actions of various cytokines and growth factors in different cell types [21]. The terminal complement components (C5 to C9) and formation of membrane attack complex have also been shown to be important for the release of proinflammatory mediators, but could point to nonlethal cell signaling and induction of cell proliferation [22, 23]. Active complement proteins have been associated with mammalian preovulatory follicular fluid [24] and uterus [25]. Both complement factor B and complement C3 mRNA have been detected in mouse uterus, but not ovary, and gene expression, particularly for

C3, is significantly increased by estrogen [26]. The complement C4 identified in Table 1 has 44% identity with carp complement C4A, but also shares approximately 25% identity with trout complement C3A. Interestingly, at least three different C3 molecules exist in trout serum, each possessing distinct binding specificities [27]. The specific roles of these various immune effectors in the developing piscine ovary, as well as in postovulatory stages as evidenced by mammalian investigations, need to be elucidated.

Coexpression of genes important in follicular maturation events

One interesting feature of this microarray analysis was the capture of the expression of a number of transcripts whose roles are intimately connected. This study revealed the transcription at steady-state levels of various cell division regulators (cdc2 and cyclin B) and ubiquitin-mediated proteolysis components (ubiquitin-conjugating enzyme E2-23 and cyclin-selective ubiquitin carrier E2-C) that selectively mark and degrade these factors (Table 4). Furthermore, the expression of the enzyme carbonyl reductase/20β-hydroxysteroid dehydrogenase (20β-HSD) was also concurrently expressed at these levels. The expression of 20β-HSD marks a steroidogenic shift in post-vitellogenic follicles from the production of estradiol-17β to synthesis of a progesterone derivative, 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP) [28]. In post-vitellogenic follicles, these changes indicate the end of rapid oocyte growth associated with vitellogenesis in response to estradiol and the start of a period of oocyte maturation influenced by a maturation promoting factor (MPF). 17α, 20β-DP exerts its action through oocyte membrane receptors to activate the formation of a complex of the two components of the MPF, cdc2 and cyclin B [28]. Post-vitellogenic oocytes (arrested in prophase) require active MPF for resumption of meiotic maturation and during meiotic arrest at the MII stage to become fertilizable [29].

It is possible we have captured a small glimpse of these processes at the gene expression level. Our work does not indicate whether or not each of these transcripts are translated in these

tissues at this stage of development. It could also be that the concurrent expression of cdc2, cyclin B and 20β-HSD (and presumably 17α, 20β-DP) is an indicator of somatic (follicle) cells undergoing mitotic divisions. Cell cycle transitions may be controlled by regulation of the ubiquitin carrier and cyclin ligase destruction machinery. To date there are no reports precisely detailing cDNA expression of each ubiquitin-proteasome component in piscine follicular cells, but the presence of some of this proteolytic machinery have been isolated from goldfish oocytes [30, 31]. It is also known that the cyclin B transcript is present in goldfish and zebrafish immature oocytes, but it is not translated until later when the oocyte meiotic maturation phase is initiated [28, 32]. It is therefore possible that similar post-transcriptional controls, as well as other regulatory constraints [see 33, 34], are placed on the transcripts that encode the proteolytic machinery that selectively degrade cyclins. The culmination of expression of this particular group of transcripts points to an interesting stage of salmon ovarian development which could, when coupled with immunodetection, lead to a greater understanding of the machinery involved in controlling mitosis and meiosis in immature and preovulatory follicles.

Expression of RBP in salmon ovary and testis

This is the first presentation of strong evidence for retinol-binding protein (RBP) gene expression in the piscine ovary (Table 1, Fig. 1). Reports for other teleosts indicate only weak, if any, expression of RBP in ovary [14, 15]. In fact, we observed RBP cDNA expression in immature (data not shown), normal (Fig. 1) and precocious (Table 1) tissues. Locally expressed RBP may serve to deliver retinol to the developing oocyte. The metabolites of the retinol could then be utilized during embryogenesis. It is also possible that delivery of retinol to the ovary from the liver (the major vertebrate storage site of retinol) is by a more general carrier such as with vitellogenin, albumin or low-density lipoproteins. Once in the ovary, then RBP may be required for transport of retinol to specific cell-types to participate in ovarian maturation. In support of this argument, expression of RBP in granulosa cells [35] and Sertoli cells [36] of the

rat has been demonstrated. To date, a complete understanding of how retinol and other nutritional and regulatory substances are deposited in the oocyte yolk has not been elucidated [14, 15].

Presence of uniquely-expressed genes in salmon ovary and testis

We also report the expression of cDNAs that encode the common glycoprotein-α (Cgα) subunit, as well as the luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH) β-subunits in the salmonid ovary. LH, FSH and TSH each share the Cgα subunit and acquire their unique attributes by heterodimeric binding through the hormone-specific β-subunits. These glycoprotein hormones are more commonly associated with expression and synthesis in the pituitary, therefore detection of their hybridizations to the array throughout ovarian development was unexpected (Table 6). Expression of these cDNAs were further demonstrated in ovarian and testicular cDNAs at different developmental stages by PCR (Fig. 1). These findings are also supported by mammalian investigations that demonstrated FSH expression in both ovary [37] and testis [38]. Although evidence exists for expression of both the Cgα and LHβ subunits in the rat testis [39], there are no corresponding reports for LHβ expression in the mammalian ovary. Therefore this report appears to be the first to indicate the potential for synthesis of both Cgα and LHβ subunits in the ovary for any species. The lack of any discernible mRNA for any of these transcripts in the unfertilized egg, as well as expression in the testes (except TSH), implicates these molecules as serving specific functions in the gonads rather than production as agents for subsequent embryogenesis.

It is known that hypothalamic GnRH controls and modulates the release of LH and FSH in the pituitary, and that GnRH synthesis occurs in both the ovary and testis [40]. Unfortunately, the microarray employed here did not contain any preproGnRH cDNA elements. However, expression of a prepro-thyrotropin-releasing hormone (the hypothalamic activator of TSH) was observed throughout the study (Table 2). Investigations to determine the physiological roles of

each of the glycoprotein hormones, as well as their activators and receptors within the gonad, are clearly required in piscine and mammalian models.

In conclusion, we have shown the utility of using microarrays to identify genes important in the development and maturation of the trout ovary. Our salmonid-gene specific microarray analysis revealed changes that occur in the expression of genes involved in cellular organization and modeling, immunoregulation, cell-cycling, as well as other areas of interest. This study enabled the tracking of specific cDNA expressions that potentially mark a crucial phase in follicular maturation. Microarrays can also serve as useful tools to detect unexpected tissue-specific expression of genes.

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Figure Legend

Reverse transcriptase PCR validation of glycoprotein subunit and retinol-binding protein (RBP) cDNA expression in trout ovaries and testes during different stages of development. Integrity of each cDNA used was confirmed by control PCR using ubiquitin primer set. For each gene-specific PCR experiment a negative control with no template was included. The strongest marker band indicates a fragment length of 500 bp.

Table 1. Genes upregulated in precocious ovary relative to normal ovary.

EST acc.	Gene name of top BLASTX hit (accession number; species)	Length (% ID)	E-value	June		August		October	
				MFC	SEM	MFC	SEM	MFC	SEM
CA063640	CR2 receptor (CAA68674; *Homo sapiens*)	236 (29.2%)	1.9E-18	43.04	9.97	16.71	2.82	22.85	4.59
CA770793	Lysozyme G (P00717; *Cygnus atratus*)	148 (64.8%)	0	23.73	10.68	1.91	0.52	9.26	1.69
CA060167	3beta-hydroxy-Delta5-steroid dehydrogenase (S48678; *O. mykiss*)	202 (99.5%)	0	18.04	3.67	6.14	0.60	6.58	0.53
CA053039	similar to procollagen C-endopeptidase enhancer 2 (BG935263; *S. salar*)	152 (93.4%)	0	15.96	2.19	3.36	0.92	26.60	3.21
CB517381	similar to pancreatic elastase IIIA precursor (XP_031238; *Homo sapiens*)	146 (52.0%)	9.8E-28	14.17	6.49	2.92	0.69	15.08	3.08
CA037998	beta-2-glycoprotein I precursor (P26644; *Rattus norvegicus*)	94 (40.4%)	1.2E-16	11.65	2.76	3.58	0.94	9.69	1.31
CA055134	adipose differentiation-related protein (Q9TUM6; *Bos taurus*)	54 (74.0%)	3.1E-16	10.26	5.38	1.47	0.68	3.11	0.77
CA057941	type I keratin S8 (CAC45059; *O. mykiss*)	131 (99.2%)	0	10.06	2.17	5.22	0.84	27.09	1.90
CA039349	retinol-binding protein (AAB24973; *Oncorhynchus mykiss)*	136 (94.8%)	0	9.53	1.17	2.89	0.19	7.51	0.51
CB505474	G-protein signaling regulator 5 homolog (JC7228; *Xenopus sp.*)	182 (69.7%)	3.6E-62	8.69	6.25	2.47	0.46	5.21	1.37
CB487033	envelope protein (AAL78047; *Danio rerio*)	103 (50.4%)	4.5E-18	8.35	1.61	2.45	0.10	6.67	0.32
CA040481	alpha 3 type I collagen (AB008374; *O. mykiss*)	217 (93.0%)	0	7.82	0.90	1.84	0.21	8.25	0.96
CB488532	fatty acid binding protein (H-FABP) (AAB53643; *Oncorhynchus mykiss*)	106 (100%)	0	7.34	1.36	3.52	0.31	9.40	0.79
CA038626	putative membrane protein (AAK01372; *Carassius auratus*)	190 (42.6%)	9.9E-34	7.19	1.50	3.57	0.33	3.22	0.42
n/a	ovulatory protein-2 precursor (AAB63598; *Salvelinus fontinalis*)	132 (65.9%)	0	6.44	0.37	8.63	0.97	28.49	1.93
CA057650	serine protease-like protein (AF005026; *Salvelinus fontinalis*)	539 (95.9%)	(n) 0	6.30	1.43	2.20	0.14	10.28	1.36
CA045301	h1-calponin alpha (AAB01453; *Mus musculus*)	78 (74.3%)	1.5E-28	5.81	4.03	1.62	0.81	4.99	1.52
CA038796	similar to spleen Class II histocompatibility antigen (BG935727; *S.salar)*	257 (98.4%)	0	5.72	0.97	1.40	0.26	7.64	1.13
CA037945	similar to spleen clone SS1-1027 (BG936642; *S.salar*)	634 (98.8%)	(n) 0	5.58	0.75	2.19	0.27	16.69	1.67
n/a	MHC-Sasa class II B (X70166; *S.salar*)	270 (98.1%)	0	5.41	0.74	1.33	0.26	8.63	0.59
CB494225	similar to spleen Tc1-like transposase (BG935785; *S.salar*)	148 (91.2%)	0	5.41	0.95	1.89	0.44	10.02	1.17
CB515225	esterase D (BAA92850; *Sus scrofa*)	149 (81.2%)	8.1E-72	5.37	1.99	2.32	0.85	6.47	1.99
CA045208	lysozyme (AAG12207; *S.salar*)	104 (97.1%)	0	5.23	0.44	1.41	0.14	3.33	0.23
CA038371	prostaglandine D synthase (AAG30028; *Oncorhynchus mykiss*)	115 (92.1%)	0	5.12	0.45	5.76	1.11	8.84	0.39
CA042513	signal sequence receptor alpha chain (I51332; *O.mykiss*)	235 (96.1%)	0	5.08	1.11	1.71	0.14	2.27	0.31
CA055171	similar to spleen thymosin beta (BG936034; *S.salar*)	225 (92.8%)	0	5.02	0.92	2.30	0.17	5.98	0.64

CA043979	aldolase B (AAD11573; *Salmo salar*)	186 (79.0%)	0	4.94	0.48	2.30	0.37	4.08	0.69
CB516043	interleukin 13 receptor alpha-2 (AAL26927; *Oncorhynchus mykiss*)	117 (95.7%)	4.5E-65	4.79	1.44	3.22	0.58	10.33	2.07
CA044030	type II keratin E1 (CAC45056; *Oncorhynchus mykiss*)	64 (90.6%)	7.3E-27	4.72	0.96	2.20	0.19	6.72	0.47
CA060011	barrier to autointegration factor (NP_003851; *Homo sapiens*)	83 (53.0%)	2.4E-19	4.63	0.79	1.77	0.38	6.70	0.27
n/a	K18, simple type I keratin (Y14289; *O.mykiss*)	510 (91.7%)	(n) 0	4.59	0.13	2.71	0.10	6.96	0.33
CA039257	haptoglobin fragment 1 (AAG30004; *Oncorhynchus mykiss*)	88 (86.3%)	3.8E-37	4.59	3.03	1.96	0.28	2.29	0.32
CB503730	CCAAT/enhancer binding protein, delta (NP_571962; *Danio rerio*)	136 (57.3%)	8.3E-35	4.58	0.90	2.91	1.02	4.09	0.70
CA048079	secreted protein, acidic, rich in cysteine (SPARC) (U25721; *O.mykiss*)	267 (88.0%)	(n) 0	4.30	2.10	1.25	0.35	4.04	0.37
CB514674	hemoglobin IV alpha chain (S03995; *O.mykiss*)	142 (97.1%)	3.0E-77	4.16	0.51	1.95	0.09	16.45	1.64
CA044716	sodium and chloride-dependent transporter NTT4 (CAC19682; *H.sapiens*)	164 (79.2%)	0	4.11	0.16	3.37	0.16	6.09	0.20
CA770624	simple type II keratin K8b (S2) (X92522; *Oncorhynchus mykiss*)	300 (92.0%)	(n) 0	4.09	0.36	2.36	0.23	8.36	0.65
CA050852	immunoglobulin light chain precursor (AAD38362; *Anarhichas minor*)	175 (65.7%)	0	4.09	1.71	0.29	0.20	2.16	0.82
CA045730	alpha-globin (CAA65949; *Salmo salar*)	143 (64.3%)	0	4.07	0.17	2.01	0.17	16.40	1.21
CA064012	beta-globin (CAA65945; *Salmo salar*)	124 (94.3%)	0	3.90	0.19	2.24	0.13	15.57	1.64
CA064459	collagen alpha 2(I) chain precursor (O93484; *O.mykiss*)	204 (98.0%)	0	3.89	0.21	1.82	0.12	9.23	0.96
CA039664	similar to kidney proteoglycan core protein (BG933846; *S.salar*)	339 (87.6%)	(n) 0	3.88	1.61	2.02	0.80	5.81	1.95
CA051812	hemoglobin beta chain (S41625; *S.salar*)	148 (100%)	0	3.86	0.19	2.23	0.11	18.44	1.42
CA767831	simple type II keratin K8a (S1) (AJ272373; *O.mykiss*)	328 (91.4%)	(n) 0	3.65	0.44	2.52	0.29	6.79	0.78
CA064377	complement C4A (BAB03284; *Cyprinus carpio*)	129 (44.1%)	4.7E-27	3.63	1.54	1.50	0.65	2.76	0.71
CA043934	selenoprotein Pa (AAG53688; *Danio rerio*)	69 (56.5%)	1.8E-18	3.39	0.26	1.82	0.10	3.96	0.10
CB494196	chaperonin subunit 3 (gamma) (NP_033966; *Mus musculus*)	104 (85.5%)	2.5E-44	3.33	0.17	2.09	0.10	12.69	2.37
CA050537	cathepsin S (NP_067256; *Mus musculus*)	50 (72.0%)	3E-19	3.28	0.84	1.46	0.26	7.56	3.41
CA057815	tissue factor pathway inhibitor 2 (NP_006519; *Homo sapiens*)	106 (43.3%)	1.7E-18	3.26	0.90	2.16	0.44	3.78	1.86
CA038817	L-plastin (AAD40680; *Danio rerio*)	93 (93.5%)	2.0E-43	3.23	0.76	1.18	0.35	5.75	1.24
CA061849	pigment epithelium-derived factor precursor (Q95121; *Bos taurus*)	112 (40.1%)	2.8E-23	3.17	0.67	1.07	0.29	3.92	0.39
CA039091	similar to ganglioside expression factor 2 (BG934586; *S.salar*)	157 (97.4%)	0	3.14	1.61	2.30	1.05	6.61	2.80
CA038599	procathepsin L (AAK69706; *Oncorhynchus mykiss*)	176 (53.4%)	0	3.12	1.14	1.54	0.25	8.59	0.70
CB494346	alpha-1 enolase-1 (AAG16310; *Salmo trutta*)	136 (86.0%)	0	3.09	0.33	2.97	0.46	4.62	0.66
CA052765	inhibitor of DNA binding 6 (NP_571320; *Danio rerio*)	84 (85.7%)	5.1E-34	3.09	0.75	0.89	0.23	3.51	0.65
CA054912	nidogen (enactin) (NP_002499; *Homo sapiens*)	80 (56.2%)	7.1E-17	2.93	0.39	2.79	0.41	9.71	1.83
CA045492	ictacalcin (Q91061; *Ictalurus punctatus*)	72 (68.0%)	2.3E-21	2.92	0.84	1.90	0.37	8.00	1.00
CA049855	complement component C7 (BAA88899; *Paralichthys olivaceus*)	205 (51.2%)	0	2.72	0.90	0.76	0.24	2.22	0.40

CA770897	Id1 protein (Y08368; *Oncorhynchus mykiss*)	524 (94.0%)	(n) 0	2.63	0.80	1.41	0.27	3.02	0.57
CA769643	L2BP1 (BAA83101; *Rattus norvegicus*)	85 (50.5%)	1.3E-17	2.59	1.08	1.58	0.83	3.96	0.67
n/a	ubiquinol--cytochrome-c reductase cytochrome b (T09959; *S.salar*)	157 (94.9%)	0	2.54	0.33	1.62	0.17	3.74	0.20
CA039139	similar to kidney SK1-0159 keratin type II (BG933881; *S.salar*)	217 (95.8%)	(n) 0	2.50	0.68	2.28	0.43	6.13	0.82
CA038518	cysteine proteinase precursor (AAF19631; *Myxine glutinosa*)	52 (73.0%)	5.8E-18	2.49	0.80	3.54	2.16	9.43	2.59
CB505045	integral membrane protein 2B (O42204; *Gallus gallus*)	53 (66.0%)	3.7E-16	2.44	0.48	1.73	0.28	3.32	0.85
CA040432	calpain 7; calpain like protease (NP_055111; *Homo sapiens*)	93 (67.7%)	1.0E-34	2.42	0.57	1.01	0.19	2.20	0.34
CA047156	similar to liver SL1-0936 elongation factor 1A (BG935574; *S.salar*)	364 (98.6%)	(n) 0	2.37	0.22	1.52	0.12	2.32	0.14
CA040031	actin-related protein complex 1b (NP_062162; *Rattus norvegicus*)	186 (88.1%)	0	2.35	0.38	1.99	0.22	2.76	0.47
CA039126	integral membrane protein 2B, ATPase domain (BG935677; *S.salar*)	169 (96.4%)	(n) 0	2.35	0.83	1.45	0.23	4.61	1.38
CA770776	liver cDNA clone SL1-0009 (BG934743; *S.salar)*	542 (99.8%)	(n) 0	2.32	0.74	2.22	0.62	3.26	0.70
CA043872	prostaglandin dehydrogenase (AAF81098; *Papio hamadryas*)	156 (54.4%)	9.8E-45	2.30	1.10	1.70	0.81	2.16	0.80
CA062039	retinol dehydrogenase type II (P50170; *Rattus norvegicus*)	139 (49.6%)	1.1E-32	2.18	0.77	0.99	0.18	2.26	0.52
CA037588	alpha1-microglobulin/bikunin protein (AAA72048; *S.salar*)	140 (84.2%)	0	2.17	0.36	0.48	0.12	5.42	0.22
CA056813	cathepsin K precursor (Q9GLE3; *Sus scrofa*)	69 (72.4%)	2.0E-24	2.16	1.79	2.03	0.52	16.53	4.74
CB492389	alpha-globin IV (BAA13534; *Oncorhynchus mykiss*)	143 (100%)	8.9E-80	2.13	0.19	1.49	0.10	11.97	1.65
CA042638	immunoglobulin light chain F class (AAA82596; *Ictalurus punctatus*)	111 (65.7%)	5.5E-37	2.13	0.71	0.39	0.12	2.05	0.20
CB513579	ependymin precursor (P38528; *Cyprinus carpio*)	215 (38.6%)	1.7E-28	2.12	0.19	1.31	0.10	6.22	0.36
CB487237	diazepam binding inhibitor (P07107; *Bos taurus*)	72 (70.8%)	5.7E-24	2.09	1.06	2.34	0.48	4.45	1.06
CA039481	transducer of ERBB-2 (AF266238; *Gillichthys mirabilis*)	157 (94.9%)	(n) 0	2.07	0.18	2.47	0.21	2.31	0.29
CA052137	SH3-domain GRB2-like 2 (NP_003017; *Homo sapiens*)	95 (81.0%)	3.9E-43	1.92	0.52	2.17	0.29	0.61	0.46
CA039058	apolipoprotein CII (AAG11410; *Oncorhynchus mykiss*)	41 (92.6%)	9.5E-17	1.89	0.99	2.79	0.69	2.12	0.43
CA055296	smooth muscle protein SM22 homolog (A60598; *Mus musculus*)	100 (78.0%)	2.6E-38	1.72	0.29	1.13	0.14	4.10	0.39
CA051843	peripheral benzodaizepine receptor (JE0149; *Homo sapiens*)	101 (65.3%)	2.9E-35	1.72	0.21	1.35	0.11	2.67	0.34
CA037686	similar to liver SL1-0424 precerebellin-like protein (BG935115; *S.salar*)	153 (96.7%)	(n) 0	1.69	0.62	2.94	0.39	6.38	1.54
CB493525	novel member of chitinase family (BAA86981; *Homo sapiens*)	123 (57.7%)	1.4E-30	0.85	0.55	3.14	1.60	5.11	2.19

Table 2. Genes downregulated in precocious ovary relative to normal ovary.

EST acc.	Gene name of top BLASTX hit (accession number; species)	Length (% ID)	E-value	June		August		October	
				MFC	SEM	MFC	SEM	MFC	SEM
CB486682	alpha-2-macroglobulin-1 (BAA85038; *Cyprinus carpio*)	106 (49.0%)	1.0E-19	5.19	1.19	5.28	1.06	9.69	1.01
CA038598	alpha-2-macroglobulin-3 (BAA85040; *Cyprinus carpio*)	183 (51.9%)	0	5.09	1.06	5.24	1.06	8.43	1.67
CA038906	similar to stonustoxin beta subunit or butyrophilin (BG936046; *S.salar*)	116 (94.8%)	(n) 0	4.97	1.89	8.13	0.52	2.71	0.33
CB486276	putative sex-lethal interactor homolog (BAB23761; *Mus musculus*)	77 (63.6%)	8.9E-25	4.83	0.33	4.11	0.36	8.40	0.87
CA036724	ovarian cysteine protease inhibitor (AAK00216; *Salvelinus fontinalis*)	88 (72.7%)	1.8E-35	4.52	0.78	1.57	0.22	4.98	0.20
CA043001	spleen clone similar to other reported ESTs (BG935980; *S.salar*)	193 (87.5%)	(n) 0	4.46	0.21	1.48	0.23	2.37	0.25
CB486643	unknown protein (AAH04641; *Mus musculus*)	113 (77.8%)	7.0E-45	4.39	0.57	2.15	0.27	4.11	0.27
CA056930	hypothetical protein XP_005578 (XP_005578; *Homo sapiens*)	203 (75.3%)	0	4.30	0.32	1.51	0.30	3.58	0.23
CA038470	similar to copper transport protein ATOX1 (BE518530; *S.salar*)	318 (98.4%)	(n) 0	3.86	0.54	2.16	0.35	3.91	0.24
CB491261	alveolin (BAA90750; *Oryzias latipes*)	110 (62.7%)	9.4E-37	3.68	0.15	2.23	0.09	4.67	0.30
CA037585	thrombin B chain variant 1 (AAG30034; *Oncorhynchus mykiss*)	117 (96.5%)	0	3.58	0.60	2.52	0.35	3.59	0.52
CB486763	tissue inhibitor of metalloproteinase 2 (AAF21942; *Canis familiaris*)	107 (57.9%)	5.5E-26	3.52	0.22	3.81	0.27	8.93	0.59
CA050082	spleen cDNA clone SS1-0134 (BG935820; *S.salar*)	590 (97.2%)	(n) 0	3.41	0.58	6.16	0.85	2.13	0.46
CA041894	alpha-1 enolase-1 (AAG16310; *Salmo trutta*)	192 (83.3%)	0	3.39	0.48	1.62	0.26	2.70	0.14
CA060884	cyclin-E binding protein 1 (XP_003492; *Homo sapiens*)	141 (44.6%)	1.9E-31	3.38	0.30	1.15	0.31	1.24	0.29
CB486079	somatic lipoprotein receptor (CAA05874; *Oncorhynchus mykiss*)	74 (98.6%)	9.4E-41	3.34	0.60	2.37	0.31	2.86	0.14
CB517677	suppression of tumorigenicity 5 (CAC38112; *Mus musculus*)	205 (94.1%)	9.0E-110	3.31	0.14	1.66	0.12	5.04	0.66
CB513675	annexin A3 (NP_038498; *Mus musculus*)	192 (55.2%)	1.5E-50	3.27	1.41	1.46	0.28	4.22	0.95
CB487951	chorion protein (CAA63709; *Sparus aurata*)	134 (62.6%)	1.4E-45	3.04	0.21	2.14	0.10	5.74	0.45
CA041403	hypothetical protein (BAB64521; *Macaca fascicularis*)	223 (80.7%)	0	2.96	0.61	1.65	0.25	2.48	0.16
CB487789	similar to zinc finger protein ZFP235 (BAB30369; *Mus musculus*)	67 (53.7%)	1.3E-31	2.95	0.20	1.27	0.10	3.00	0.21
CA060220	similar to di-N-acetylchitobiase (AAH22594; *Mus musculus*)	85 (60.0%)	7.6E-27	2.91	0.18	1.81	0.12	2.03	0.08
CA044472	MHC class I (AAA49602;*S.salar)*	118 (87.2%)	0	2.90	0.88	2.67	0.60	2.72	0.63
CB491304	ZPC domain containing protein 5 (AAD38910; *Oryzias latipes*)	110 (42.7%)	8.6E-16	2.79	0.29	2.03	0.14	4.61	0.23
CA044434	integral type I protein (NP_031390; *Homo sapiens*)	132 (68.1%)	0	2.66	0.93	1.73	0.18	3.04	0.31
CA050826	putative homolog to CGI-35 protein (BAB22857; *Mus musculus)*	98 (93.8%)	0	2.66	1.12	1.27	0.33	3.08	0.59

CA038790	antithrombin (CAB64714; *Salmo salar*)	141 (100%)	0	2.64	0.37	2.48	0.17	2.74	0.22
CA039214	c-myc binding protein (XP_001357; *Homo sapiens*)	103 (65.0%)	1.2E-32	2.55	0.24	2.03	0.21	3.24	0.33
n/a	oocyte zinc finger protein XLCOF8.4 (P18753; *Xenopus laevis*)	103 (48.5%)	5.9E-20	2.52	0.24	1.38	0.15	2.35	0.16
CB487887	Lsm1 protein (NP_055277; *Homo sapiens*)	113 (82.3%)	1.7E-38	2.50	0.39	1.63	0.15	3.44	0.24
CB492395	gammaN-crystallin (AAL40969; *Mus musculus*)	149 (70.4%)	7.8E-68	2.44	0.30	6.80	2.12	2.28	0.28
CA053157	stathmin (CAA46450; *Gallus gallus*)	95 (80.0%)	2.4E-28	2.43	0.60	1.29	0.13	5.62	0.91
CB488409	Cu/Zn-superoxide dismutase (AF469663; *Oncorhynchus mykiss*)	122 (99.1%)	(n) 3.6E-60	2.42	0.43	1.22	0.15	3.46	0.21
CB486721	vitellogenin receptor (CAA05873; *Oncorhynchus mykiss*)	184 (100%)	0	2.40	0.41	2.22	0.17	2.60	0.15
CB511750	IHABP (AAN10161; *Takifugu rubripes*)	167 (38.9%)	5.3E-19	2.40	0.58	1.91	0.18	2.10	0.16
n/a	vitelline envelope protein gamma (AAF71260; *Oncorhynchus mykiss*)	137 (85.4%)	0	2.38	0.43	1.29	0.24	3.76	0.36
CB486193	nucleoplasmin (P05221; *Xenopus laevis*)	131 (48.8%)	2.4E-31	2.37	0.24	1.69	0.10	2.99	0.18
CB502471	PKCI-Z-related protein (AAN16460; *Taeniopygia guttata*)	120 (74.1%)	1.9E-53	2.37	0.35	1.83	0.21	2.70	0.08
CA043347	B-cell translocation protein 1 (NP_001722; *Homo sapiens*)	167 (57.4%)	0	2.28	0.53	1.52	0.16	3.57	0.17
CB486365	chorion proteic component (NP_571771; *Danio rerio*)	157 (36.3%)	6.0E-25	2.28	0.14	2.50	0.24	3.41	0.58
CA037803	similar to S. pombe dim1+ (NP_006692; *Homo sapiens*)	48 (93.7%)	2.1E-21	2.25	0.17	1.08	0.11	2.23	0.08
CA049444	histone cell cycle interacting protein 5 (BAB22965; *Mus musculus*)	138 (53.6%)	1.6E-27	2.25	0.33	1.08	0.27	2.81	0.15
CA059480	rhamnose-binding lectin WCL3 (BAB83629; *Salvelinus leucomaenis*)	104 (70.1%)	1.3E-40	2.24	0.17	1.98	0.08	4.21	0.25
CA053954	eukaryotic translation initiation factor 3 (XP_034519; *Homo sapiens*)	84 (89.2%)	3.5E-40	2.24	0.14	2.00	0.08	2.08	0.12
CA038888	ISCU2 (AAG37428; *Homo sapiens*)	54 (94.4%)	4.5E-24	2.21	0.18	1.23	0.09	2.50	0.09
CA047477	RING finger protein (AAD30147; *Homo sapiens*)	56 (100%)	6.0E-31	2.20	0.23	1.43	0.14	2.59	0.31
CB487936	ZPA domain containing protein (AAD38904; *Oryzias latipes*)	216 (42.1%)	9.4E-44	2.20	0.10	2.47	0.08	2.83	0.38
CB488242	egg envelope glycoprotein ZP3 (AAD53946; *Carassius auratus*)	151 (53.6%)	8.0E-44	2.17	0.12	1.53	0.08	2.42	0.09
CB491281	unknown protein for MGC:19163 (AAH13499; *Mus musculus*)	144 (56.2%)	8.3E-36	2.17	0.11	1.88	0.08	4.07	0.07
CA061778	cytochrome P450 monooxygenase (AAC28310; *O. mykiss*)	97 (90.7%)	0	2.15	0.25	2.98	0.11	3.72	0.09
n/a	beta crystallin A2 (P55164; *Gallus gallus*)	92 (75.0%)	0	2.15	0.50	0.93	0.60	2.13	0.37
CA045465	cytoplasmic dynein light chain (NP_525075; *Drosophila melanogaster*)	89 (97.7%)	0	2.13	0.48	1.14	0.09	2.42	0.15
CA057552	nonclathrin coat protein zeta1-COP (BAA92783; *Danio rerio*)	78 (94.8%)	8.4E-34	2.12	0.13	1.32	0.08	2.29	0.14
CB487230	RNA binding protein 42Sp43 (AAD38911; *Oryzias latipes*)	66 (62.1%)	1.4E-19	2.11	0.10	1.84	0.08	3.17	0.15
n/a	similar to annexin A2 (AAH09564; *Homo sapiens*)	124 (73.3%)	0	2.09	0.13	2.15	0.10	1.35	0.11
CA059808	growth arrest specific (NP_061343; *Mus musculus*)	161 (70.8%)	0	2.06	0.22	2.02	0.16	2.60	0.18
CB513932	B-cell receptor-associated protein 37 (XP_110594; *Mus musculus*)	194 (75.7%)	6.2E-74	2.05	0.13	1.44	0.16	2.13	0.07
CB516471	unnamed protein product (BAA95095; *Mus musculus*)	197 (69.5%)	1.2E-77	2.05	0.27	1.46	0.11	4.47	0.18
CA040151	thyroid receptor interacting protein 15 (NP_004227; *Homo sapiens*)	130 (97.6%)	0	2.03	0.35	0.80	0.35	4.47	0.88

CA055556	HSPC274 protein (XP_054678; *Homo sapiens*)	119 (76.4%)	2.7E-37	2.00	0.25	3.09	0.61	2.85	0.32
CA047451	transposase (CAB51371; *Pleuronectes platessa*)	116 (56.0%)	1.7E-29	2.00	0.32	1.93	0.20	3.97	0.17
CB486367	rhamnose binding lectin STL3 (BAA92257; *Oncorhynchus mykiss*)	101 (97.0%)	0	1.95	0.22	1.69	0.16	3.52	0.12
CB487976	ZPC domain containing protein 2 (AAD38907; *Oryzias latipes*)	180 (56.1%)	0	1.92	0.10	1.48	0.08	2.86	0.25
n/a	COP9 subunit 3 (NP_003644; *Homo sapiens*)	89 (83.1%)	8.0E-35	1.92	0.24	1.54	0.17	2.03	0.12
CB517349	prepro-thyrotropin-releasing hormone (BAB88661; *O. nerka*)	201 (97.0%)	3.8E-113	1.72	0.23	2.54	0.19	6.66	2.47
CB486697	vitelline envelope protein alpha (AAF71258; *Oncorhynchus mykiss*)	172 (54.0%)	0	1.55	0.32	1.31	0.23	2.71	0.62
CA060171	Mx3 protein (AAB40996; *S. salar*)	55 (100%)	1.0E-25	1.22	0.27	2.49	0.51	2.83	0.31
CA044877	cell death-inducing DFFA-like effector b (XP_033245; *Homo sapiens*)	70 (61.4%)	8.1E-18	1.10	0.32	4.34	0.28	2.06	0.26

Table 3. Potential immunoregulators expressed between two-fold lines for each development stage.

EST acc.	Gene name of top BLASTX hit (accession number; species)	Length (% ID)	E-value	June		August		October	
				MFC	SEM	MFC	SEM	MFC	SEM
CB516696	immunoglobulin light chain (BAB91007; *Cyprinus carpio*)	178 (61.7%)	1.6E-33	3.14	1.34	0.31	0.23	1.21	1.02
CA040242	similar to spleen T-cell antigen receptor 3' UTR (BG936592; *Salmo salar*)	164 (98.7%)	(n) 0	1.77	0.81	0.54	0.43	0.80	0.33
CA061336	macrophage receptor with collagenous structure (NP_034896; *Mus musculus*)	108 (50.9%)	1.3E-22	1.70	1.00	1.05	0.35	3.11	0.57
n/a	differentially regulated trout protein 1 (AAG30030; *Oncorhynchus mykiss*)	38 (94.7%)	3.8E-38	1.63	0.16	1.30	0.11	1.95	0.09
CA040830	cytokine receptor common gamma chain (AJ276623; *Oncorhynchus mykiss*)	316 (92.4%)	(n) 0	1.60	2.09	0.97	0.44	1.34	0.57
CA038002	**complement factor Bf-1 (AAC83699; *Oncorhynchus mykiss*)**	**60 (88.3%)**	**2.0E-28**	**1.59**	**0.37**	**1.17**	**0.26**	**1.53**	**0.35**
CA040296	MHC class I heavy chain precursor (Onmy-UBA) (AF287484; *O. mykiss*)	375 (94.4%)	(n) 0	1.57	0.33	0.84	0.10	1.28	0.10
n/a	neutrophil cytosolic factor 2 (XP_002200; *Homo sapiens*)	114 (54.3%)	3.5E-29	1.46	0.52	1.10	0.41	1.21	0.52
CA041734	similar to liver megakaryocyte stimulating factor (BG934913; *Salmo salar*)	281 (98.5%)	(n) 0	1.41	0.81	1.49	0.36	1.35	0.47
CB504350	natural resistance ass'd macrophage protein-alpha (AAD20721; *O. mykiss*)	169 (87.5%)	1.3E-83	1.40	0.46	1.16	0.25	0.68	0.36
CA039888	immunoglobulin heavy chain variable region (AAG21259; *Salmo salar*)	127 (94.4%)	0	1.40	0.17	0.52	0.08	0.79	0.09
CA049564	immunoglobulin heavy chain constant region (AAB24064; *Salmo salar*)	89 (100%)	0	1.36	0.18	0.22	0.08	1.13	0.09
CA055773	IgE binding protein (AAA41378; *Rattus norvegicus*)	105 (47.6%)	1.7E-24	1.26	0.30	1.32	0.17	1.35	0.15
CB490808	natural killer cell enhancement factor (AF250193; *Oncorhynchus mykiss*)	141 (97.8%)	(n) 0	1.25	0.27	0.82	0.19	0.73	0.17
CA044026	MHC class I (I51348; *Salmo salar*)	185 (67.0%)	0	1.12	0.74	0.44	0.17	1.07	0.14
CA052045	**complement component 7 (AAG30011; *Oncorhynchus mykiss*)**	**72 (90.2%)**	**8.5E-35**	**1.12**	**0.52**	**0.91**	**0.43**	**1.48**	**0.81**
CB493896	eosinophil chemotactic cytokine (NP_068569; *Homo sapiens*)	159 (47.7%)	3.8E-34	1.09	0.20	1.14	0.11	1.31	0.18
CA053646	endothelial monocyte-activating protein II precursor (B55053; *Homo sapiens*)	126 (77.7%)	0	1.00	0.13	1.07	0.12	1.16	0.11
CA061887	Ig heavy chain C region (A46533; *Salmo salar*)	210 (96.6%)	0	0.96	0.44	0.31	0.14	1.17	0.20
CB487123	T-cell-originated protein kinase (MAPKK-like) (NP_075698; *Mus musculus*)	156 (59.6%)	1.7E-43	0.87	0.18	1.13	0.13	0.51	0.39
CA058319	macrophage migration inhibitory factor (NP_002406; *Homo sapiens*)	114 (69.2%)	1.0E-41	0.81	0.10	0.98	0.08	0.63	0.08
CA052159	cyclooxygenase-1 (CAC10360; *Oncorhynchus mykiss*)	171 (98.8%)	0	0.80	0.11	0.88	0.08	0.52	0.07
CB506362	MHC class II alpha chain (AAC64371; *Aulonocara hansbaenschi*)	132 (64.3%)	2.2E-34	0.77	0.13	0.96	0.13	0.86	0.13
n/a	hematopoietic necrosis virus infected kidney (AU081135; *O. mykiss*)	346 (95.6%)	(n) 0	0.75	0.19	1.12	0.19	0.72	0.07
CA061789	kidney clone SK1-0644 similar to MHCII beta chain (BG934345; *Salmo salar*)	128 (98.4%)	(n) 0	0.75	0.10	0.83	0.09	0.61	0.07
CB513508	T-complex protein 1 delta subunit (CAB94911; *Gallus gallus*)	170 (84.7%)	4.6E-73	0.72	0.11	0.77	0.09	0.57	0.08

CA048625	immunoglobulin light chain variable region (CAB72437; *Oncorhynchus mykiss*)	44 (81.8%)	5.1E-16	0.61	0.20	0.93	0.13	0.51	0.13
CA058874	autoimmune infertility-related protein; 5'-nucleotidase (NP_081864; *M.musculus*)	173 (75.1%)	0	0.60	0.39	1.21	0.44	0.69	0.43
CB490770	T cell receptor beta chain variable region (AF329716; *Oncorhynchus mykiss*)	155 (96.7%)	(n) 0	0.56	0.32	0.55	0.18	0.83	0.48
CB488206	IgM heavy chain (AAB27359; *Oncorhynchus mykiss*)	77 (97.4%)	2.4E-39	0.53	0.42	0.35	0.20	0.76	0.27
CA062615	**complement component 1, q binding protein (NP_062132; *R. norvegicus*)**	**137 (70.8%)**	**0**	**0.52**	**0.12**	**0.68**	**0.10**	**0.52**	**0.08**

Table 4. Ubiquitin-proteasome components and cell division regulators expressed between two-fold lines for each development stage.

EST acc.	Gene name of top BLASTX hit (accession number; species)	Length (% ID)	E-value	June		August		October	
				MFC	SEM	MFC	SEM	MFC	SEM
CA051628	cyclin I (Q9Z2V9; *Mus musculus*)	188 (46.2%)	7.3E-29	1.64	0.50	1.32	0.30	2.04	0.37
n/a	26S proteasome regulatory complex subunit p37B (AAF08394; *D.melanogaster*)	129 (77.5%)	0	1.61	0.43	1.46	0.15	1.53	0.37
CA060109	programmed cell death 2 (XP_035702; *Homo sapiens*)	144 (53.4%)	2.3E-39	1.60	1.74	1.37	0.30	1.15	0.62
CA041389	cell division cycle 42 (GTP-binding protein, 25kD) (NP_001782; *Homo sapiens)*	157 (87.2%)	0	1.45	0.14	1.36	0.17	1.43	0.21
CB512888	similar to programmed cell death 4 (AAH26104; *Homo sapiens*)	180 (75.0%)	1.9E-32	1.33	0.24	1.34	0.16	1.26	0.13
CA055774	DNA mismatch repair gene homologue (NP_000526; *Homo sapiens*)	116 (81.0%)	0	1.32	0.77	0.96	0.49	1.46	0.98
CA060935	cell division cycle 10 (XP_011595; *Homo sapiens*)	106 (86.7%)	4.0E-32	1.25	0.28	1.24	0.13	1.16	0.19
CB488557	proteasome subunit alpha type 1 (O42265; *Gallus gallus*)	72 (84.7%)	4.3E-30	1.23	1.17	0.52	0.44	0.70	0.21
CA057676	ubiquitin-protein ligase E3 MDM2 (O42354; *Danio rerio*)	80 (83.7%)	6.4E-35	1.12	0.31	1.20	0.18	0.71	0.18
CA041252	ubiquitin carrier protein (NP_005330; *Homo sapiens*)	115 (86.9%)	0	1.11	0.39	1.02	0.32	1.02	0.28
CA045786	proteasome subunit N3 (AAK51461; *Oncorhynchus mykiss*)	161 (100%)	0	1.10	0.16	0.93	0.09	1.23	0.08
CB515955	cyclin E (NP_571070; *Danio rerio*)	225 (78.6%)	1.3E-99	1.08	0.13	0.76	0.13	1.02	0.09
n/a	proteasome 26S subunit, ATPase, 1 (NP_002793; *Homo sapiens*)	103 (96.1%)	0	1.06	0.16	0.82	0.09	1.17	0.10
CA056318	26S protease regulatory subunit 8 (TRIP1) (P47210; *Homo sapiens*)	73 (97.2%)	0	1.03	0.19	1.11	0.11	1.27	0.10
CA062877	**cdc2 (BAB13720; *Oryzias latipes*)**	**137 (87.5%)**	**0**	**1.01**	**0.10**	**1.34**	**0.11**	**0.81**	**0.09**
CA045510	NADH ubiquinone oxidoreductase subunit MWFE (NP_062316; *Mus musculus*)	69 (78.2%)	1.4E-26	0.99	0.29	1.07	0.14	1.22	0.27
CB486366	proliferation-associated 2G4, 38kD (NP_006182; *Homo sapiens*)	200 (79.0%)	0	0.93	0.22	0.56	0.11	0.61	0.11
CB512964	proteasome 26S subunit, non-ATPase, 5 (NP_542121; *Mus musculus)*	225 (65.3%)	2.5E-82	0.92	0.14	1.08	0.18	0.92	0.10
CA063336	inhibitor of apoptosis protein (Q90660; *Gallus gallus*)	144 (60.4%)	3.1E-33	0.90	0.15	0.89	0.11	0.83	0.10
CB487964	growth and transformation-dependent (fragment) (BAB26601;*Mus musculus*)	93 (53.7%)	2.3E-23	0.90	0.16	0.67	0.10	0.63	0.11
CB489182	anaphase promoting complex subunit 11 homolog (NP_079665; *Mus musculus*)	84 (90.4%)	8.4E-45	0.89	0.31	0.85	0.15	0.76	0.18
CA041303	proteasome activator subunit 1 (NP_571450; *Danio rerio*)	132 (85.6%)	0	0.87	0.12	0.63	0.09	0.90	0.09
CA062723	NADH-ubiquinone oxidoreductase 39 KD subunit (P34943; *Bos taurus*)	191 (68.5%)	0	0.86	0.34	0.65	0.22	0.86	0.25
CA038630	defender against cell death 1 (NP_001335; *Homo sapiens*)	113 (77.8%)	1.8E-44	0.85	0.11	0.99	0.08	0.64	0.08
CB487955	cyclin-dependent kinase regulatory subunit cyclin B2 (BAB17218; *O.curvinotus*)	240 (76.6%)	0	0.84	0.12	1.07	0.09	0.80	0.08
CB504502	NADH-ubiquinone oxidoreductase AGGG subunit (Q02374; *Bos taurus*)	74 (71.6%)	1.2E-28	0.83	0.15	0.80	0.11	0.46	0.16

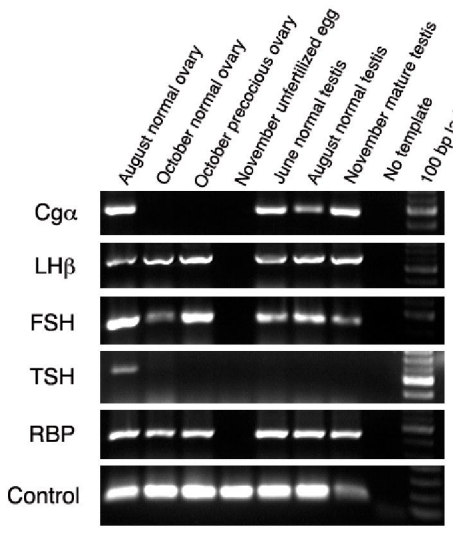
CB486616	cyclin A1 (Q92161; *Carassius auratus*)	175 (42.8%)	1.5E-27	0.82	0.13	0.89	0.12	0.60	0.08
CA051522	proteasome 28 subunit, beta; protease (NP_035320; *Mus musculus*)	133 (74.4%)	0	0.81	0.53	0.72	0.18	1.12	0.28
CA059887	ubiquitin-conjugating enzyme E2I (NP_571426; *Danio rerio*)	82 (100%)	2.4E-42	0.81	0.12	1.05	0.10	0.54	0.07
CA045997	NADH-ubiquinone oxidoreductase 13 KDA-B subunit (P23935; *Bos taurus*)	89 (64.0%)	4.8E-30	0.81	0.13	1.02	0.13	0.78	0.07
CA052514	cell division cycle 7 (NP_003494; *Homo sapiens*)	181 (55.8%)	2.8E-45	0.78	0.22	0.97	0.16	0.65	0.19
n/a	cell division protein kinase 4 (P35426; *Rattus norvegicus*)	94 (56.3%)	1.7E-25	0.77	0.14	0.98	0.09	0.92	0.10
CA060875	ras-related protein (NP_112352; *Rattus norvegicus*)	130 (93.0%)	0	0.77	0.18	0.85	0.11	0.68	0.10
CA037835	cell division cycle 42 (isoform 2), GTP-binding protein (NP_426359; *H. sapiens*)	49 (95.9%)	8.0E-20	0.76	0.21	0.97	0.10	0.65	0.11
n/a	NADH-ubiquinone oxidoreductase B8 subunit (Q02370; *Bos taurus*)	72 (66.6%)	1.0E-22	0.74	0.13	1.08	0.11	0.59	0.07
n/a	cyclin B2 (BAA89700; *Oryzias latipes*)	160 (77.5%)	0	0.74	0.51	0.87	0.39	0.78	0.33
CA042481	**ubiquitin-conjugating enzyme E2-23 (ligase) (P52483; *Mus musculus*)**	**173 (84.9%)**	**0**	**0.71**	**0.10**	**0.80**	**0.09**	**0.67**	**0.09**
CA052616	proliferating cell nuclear antigen (cyclin) (P18248; *Xenopus laevis*)	76 (92.1%)	0	0.71	0.12	0.68	0.08	0.69	0.07
CA052701	**cyclin-selective ubiquitin carrier protein E2-C (BAA85660; *C. auratus*)**	**100 (87.0%)**	**0**	**0.70**	**0.24**	**1.13**	**0.19**	**0.53**	**0.08**
CA056010	ubiquitin-conjugating enzyme 9-2 (AAG48365; *Danio rerio*)	58 (100%)	1.4E-26	0.66	0.12	1.17	0.14	0.53	0.07
CA052885	**G2/mitotic-specific cyclin B (Q92162; *Carassius auratus*)**	**161 (85.7%)**	**0**	**0.65**	**0.12**	**0.75**	**0.08**	**0.45**	**0.07**
CB516536	meiotic recombination protein REC14 (NP_075680; *Mus musculus*)	176 (89.7%)	3.9E-88	0.64	0.55	0.85	0.25	1.17	0.85
CA054038	proliferation-related protein P80 (AAF08305; *Homo sapiens)*	222 (68.9%)	0	0.62	0.30	1.34	0.28	0.80	0.20
n/a	**carbonyl reductase/20beta-HSD B (AAD20217; *Oncorhynchus mykiss*)**	**78 (96.1%)**	**9.9E-42**	**0.56**	**0.26**	**0.89**	**0.22**	**0.87**	**0.21**
CA046571	ubiquitin-conjugating enzyme E2D 3 (NP_003331; *Homo sapiens*)	147 (97.2%)	0	0.55	0.11	0.54	0.08	0.52	0.06
CA059678	component C5 of proteasome (CAA56702; *Mus musculus*)	175 (86.8%)	0	0.52	0.12	0.80	0.12	0.49	0.07
CB487801	proteasome subunit, alpha type, 4 (NP_002780; *Homo sapiens*)	135 (99.2%)	0	0.51	0.11	0.60	0.10	0.45	0.07
CA061178	similar to ubiquitin-conjugating enzyme E2E 3 (AAH16265; *Mus musculus*)	128 (95.3%)	0	0.51	0.10	0.72	0.10	0.49	0.10

Table 5. Various tissue remodeling regulators expressed between two-fold lines for each development stage.

EST acc.	Gene name of top BLASTX hit (accession number; species)	Length (% ID)	E-value	June		August		October	
				MFC	SEM	MFC	SEM	MFC	SEM
CA047512	beta-2 microglobulin (AF180478; *Salmo salar*)	613 (99.3%)	(n) 0	2.12	0.13	1.35	0.12	1.50	0.08
CA039087	elastase (serine proteinase) (1ELT; *Salmo salar*)	134 (61.1%)	0	1.25	0.77	2.86	0.87	1.31	0.32
CA054726	tissue inhibitor of metalloproteinases-2 (O42146; *Gallus gallus*)	107 (69.1%)	4.6E-43	1.07	0.22	1.30	0.17	1.04	0.09
CB498446	meprin 1 alpha (NP_037275; *Rattus norvegicus*)	120 (48.3%)	2.5E-21	1.05	0.27	0.65	0.23	3.94	0.14
CB498353	matrix metalloproteinase-2 (AB021698; *Oncorhynchus mykiss*)	695 (93.6%)	(n) 0	0.81	0.69	0.38	0.26	0.68	0.34
CA043196	elastase 4 precursor (BAA82370; *Paralichthys olivaceus*)	78 (87.1%)	1.1E-35	0.75	0.68	0.52	0.38	0.68	0.35

Table 6. Growth factors, cytokines and hormones expressed between two-fold lines for each development stage.

EST acc.	Gene name of top BLASTX hit (accession number; species)	Length (% ID)	E-value	June		August		October	
				MFC	SEM	MFC	SEM	MFC	SEM
CA045886	somatolactin (BAA01485; *Oncorhynchus keta*)	88 (97.7%)	1.4E-45	5.49	3.84	1.34	0.46	1.26	0.38
CA046686	**luteinizing hormone beta subunit (LH beta) (AB050836; *O. mykiss*)**	**176 (94.3%)**	**(n) 0**	**3.11**	**2.38**	**1.06**	**0.59**	**0.97**	**0.56**
CA048605	inducible nitric oxide synthase (AJ295231; *Oncorhynchus mykiss)*	343 (92.1%)	(n) 0	2.11	1.45	0.16	0.14	1.57	0.33
CA046811	**gonadotropin alpha subunit (Cg alpha) (AAB34358; *O. tshawytscha*)**	**70 (100%)**	**1.2E-40**	**1.33**	**0.67**	**1.29**	**0.82**	**1.00**	**0.64**
CA050841	interleukin-1 beta (AJ004821; *Oncorhynchus mykiss*)	184 (88.0%)	(n) 0	1.29	0.58	0.90	0.34	0.90	0.45
CA059491	orf within vasotocin gene (Tes1 element) (B46189; *Eptatretus stoutii*)	118 (53.3%)	0	1.07	0.15	1.13	0.08	0.92	0.08
CA049948	insulin-like growth factor I precursor (AF063216; *Oncorhynchus keta*)	113 (94.6%)	(n) 2.2E-44	1.05	0.18	1.06	0.13	0.82	0.13
CA043091	soluble angiotensin II-binding protein homolog {AAB28464; *Rattus sp.*)	90 (82.2%)	1.4E-39	0.93	0.21	1.00	0.10	0.92	0.10
CA058205	interferon, gamma-inducible protein 30 (XP_038146; *Homo sapiens*)	162 (43.2%)	1.1E-33	0.85	0.15	0.73	0.10	0.84	0.11
n/a	proopiomelanocortin (POMC) (K02614; *O.keta*)	540 (94.6%)	(n) 0	0.82	0.15	1.00	0.10	0.75	0.10
CA768663	prolactin precursor (P48096; *S. salar*)	36 (100%)	2.0E-14	0.76	0.39	1.10	0.20	0.49	0.19
CA053662	similar to mouse interferon-related protein PC4 (AAC24562; *H. sapiens*)	162 (72.8%)	0	0.73	0.23	0.85	0.16	0.80	0.11
CA055396	isotocin / neurophysin 2 precursor (JC1490; *Oncorhynchus keta*)	133 (83.4%)	0	0.63	0.38	1.24	0.23	0.74	0.24
CA047120	**follicle-stimulating hormone beta subunit (FSH) (AB050835; *O. mykiss*)**	**141 (92.1%)**	**(n) 0**	**0.59**	**0.19**	**0.98**	**0.20**	**0.62**	**0.21**
n/a	**thyrotropin beta subunit (TSH) (AF060566; *Salmo salar*)**	**262 (94.6%)**	**(n) 0**	**0.15**	**0.53**	**0.61**	**0.43**	**1.54**	**0.70**



von Schalburg; Figure 1