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# Multiple Thyrotropin β-Subunit and Thyrotropin Receptor-Related Genes Arose during Vertebrate Evolution



### Gersende Maugars<sup>1</sup>, Sylvie Dufour<sup>1</sup>, Joëlle Cohen-Tannoudji<sup>2</sup>, Bruno Quérat<sup>2</sup>\*

1 Muséum National d'Histoire Naturelle, Sorbonne Universités, Biology of Aquatic Organisms and Ecosystems (BOREA), Paris, France, Université Pierre et Marie Curie, Paris, France, Université Caen Basse Normandie, Caen, France, Unité Mixte de Recherche (UMR) 7208 Centre National de la Recherche Scientifique (CNRS), Paris, France, Institut de Recherche pour le Développement (IRD) 207, Paris, France, 2 Université Paris Diderot, Sorbonne Paris Cité, Biologie Fonctionnelle et Adaptative (BFA), Paris, France, UMR CNRS 8251, Paris, France, INSERM U1133 Physiologie de l'axe gonadotrope, Paris, France

### Abstract

Thyroid-stimulating hormone (TSH) is composed of a specific  $\beta$  subunit and an  $\alpha$  subunit that is shared with the two pituitary gonadotropins. The three  $\beta$  subunits derive from a common ancestral gene through two genome duplications (1R and 2R) that took place before the radiation of vertebrates. Analysis of genomic data from phylogenetically relevant species allowed us to identify an additional  $Tsh\beta$  subunit-related gene that was generated through 2R. This gene, named  $Tsh\beta_2$ , present in cartilaginous fish, little skate and elephant shark, and in early lobe-finned fish, coelacanth and lungfish, was lost in ray-finned fish and tetrapods. The absence of a second type of TSH receptor (Tshr) gene in these species suggests that both TSHs act through the same receptor. A novel  $Tsh\beta$  sister gene, named  $Tsh\beta3$ , was generated through the third genomic duplication (3R) that occurred early in the teleost lineage.  $Tsh\beta3$  is present in most teleost groups but was lostin tedraodontiforms. The 3R also generated a second Tshr, named Tshrb. Interestingly, the new Tshrb was translocated from its original chromosomic position after the emergence of eels and was then maintained in its new position. Tshrb was lost in tetraodontiforms and in ostariophysians including zebrafish although the latter species have two TSHs, suggesting that TSHRb may be dispensable. The tissue distribution of duplicated  $Tsh\beta s$  and Tshrs was studied in the European eel. The endocrine thyrotropic function in the eel would be essentially mediated by the classical  $Tsh\beta$  and Tshra, which are mainly expressed in the pituitary and thyroid, respectively.  $Tsh\beta 3$  and Tshrb showed a similar distribution pattern in the brain, pituitary, ovary and adipose tissue, suggesting a possible paracrine/autocrine mode of action in these non-thyroidal tissues. Further studies will be needed to determine the binding specificity of the two receptors and how these two TSH systems are interrelated.

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. Eel TSHbeta3, TSHRa and TSHRb sequences are available from EMBL database under the accession numbers LM655248-51. Coelacanth TSHbeta and TSHbeta2 and medaka TSHb3 sequences are available from EMBL database under the accession numbers (LK392305-7).

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\* Email: bruno.querat@univ-paris-diderot.fr

### Introduction

Thyroid-Stimulating Hormone (TSH) is a pituitary glycoprotein hormone responsible for the activation of the thyroid gland, playing a key role in the control of development and metabolism in mammals and other vertebrates [1]. TSH is also responsible for triggering specific developmental processes such as larval metamorphosis in amphibians [2,3], as well as larval and secondary metamorphoses in some teleost species [4–8]. In addition, TSH may participate in the modulation of various functions for example in the immune or reproductive systems, *via* pleiotropic effects and multiple target tissues of thyroid hormones [9–12].

The vertebrate pituitary glycoprotein hormones, TSH and the two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are heterodimers composed of a common  $\alpha$  subunit, and a  $\beta$  subunit that confers hormonal specificity [13]. It has recently been demonstrated [14] that the three glycoprotein

hormone  $\beta$  (GPH $\beta$ ) subunits were generated by successive duplications starting from an ancestral glycoprotein hormone  $\beta$ subunit gene  $(ancGph\beta)$  through two rounds of genomic duplications (1R and 2R) that occurred early in the evolution of vertebrates [15]. During 1R the original  $ancGph\beta$  duplicated into two paralogous genes, one of which became the evolutionary precursor of the gonadotropin  $\beta$  subunit genes (*preGth* $\beta$ ) and the other, the precursor of the  $Tsh\beta$  subunit gene (*preTsh* $\beta$ ).  $Lh\beta$  and  $Fsh\beta$  were generated next by the duplication of  $preGth\beta$  during 2**R**.  $Tsh\beta$  derived from  $preTsh\beta$  but the presence of a 2**R**-derived  $Tsh\beta$  subunit sister gene has never been demonstrated [14]. Analysis of the glycoprotein hormone related gene repertoire of the elephant shark (Callorhinchus milii) interestingly revealed the presence of two copies of  $Tsh\beta$  subunit related genes. Whether they resulted from a specific, local duplication of the  $Tsh\beta$  gene or from the conservation in cartilaginous fish of the  $Tsh\beta$  subunit

sister gene derived from the 2R, could not be determined at that time [14].

The scenario appears rather similar in extant teleosts [16] despite the specific genomic duplication (3R) that took place in this lineage [17]. A *Tsh* $\beta$  sister gene was however identified in some teleost genomes, that was shown to be derived from the 3R [14,18].

The glycoprotein hormones exert their action by interacting with specific and evolutionarily related G protein-coupled receptors. The glycoprotein hormone receptors (GPHR) are characterized by a large extracellular hormone-binding domain composed of a leucine rich domain connected to a seventransmembrane domain by a hinge region [19]. If a second TSH related hormone is present in some species, it seems logical to assume that it acts through a novel receptor, as suggested by the recent characterization of a second TSHR-like gene in some teleost species [20,21]. However, it was not clearly demonstrated whether this second TSHR was generated at the 3R or by a specific duplication early in the teleost lineage.

Whether additional  $Tsh\beta$  subunit genes and Tshr genes were derived from 2R and 3R were questions we addressed in this study. We took advantage of the recently released genomic data from several species that have a phylogenetically relevant position among vertebrates: two representatives of cartilaginous fish (chondrichthyes), a group that preceded the divergence of rayfinned fish (actinopterygies) and lobe-finned fish (sarcopterygies), the elephant shark, an holocephalan for which a new version of the genomic assembly was recently released [22] and the little skate (Leucoraja erinacea), an elasmobranch; the spotted gar (Lepisos*teus oculatus*) a ray-finned fish representative that took root before the teleost radiation and the 3R [23] and the coelacanth (Latimeria chalumnae), a lobe-finned fish that appeared just prior to lungfish [24], the lungfish group being the sister group of tetrapods [25]. The genomes of a number of teleost fish species including the eels (Anguilla anguilla and A. japonica), representatives of the basal group of Elopomorphs [26-29] also recently released, were searched for the 3R generated  $Tsh\beta$  related subunit and for a *Tshr* related gene. The tissue distribution of the two  $Tsh\beta$ and the two *Tsh receptors* was analysed in the European eel.

### **Materials and Methods**

All aspects of animal care and experimentation were in accordance with the Ethic committee of the Museum National d'Histoire Naturelle and approved by the Institutional Animal Care and Use Committee of the Animal Protection and Health, Veterinary Services Direction, Paris, France.

#### Identification of vertebrate $Tsh\beta$ and Tshr sequences

Blast analyses [30] were performed on-line using protein as query (tBlastn) on NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi), Ensembl (http://www.ensembl.org/Multi/blastview), DDBJ (http://blast.ddbj.nig.ac.jp/), as well as on web sites for little skate (Skatebase: http://skatebase.org/skateBLAST and elephant shark (http://esharkgenome.imcb.a-star.edu.sg/blast/). Eel sequences were identified from European and Japanese eel genomes available on the website Eel Genome of ZF-Genomics (http:// www.zfgenomics.org/sub/eel) in addition to the assembly available in NCBI, using the CLC BIO software (Qiagen, Denmark).

Protein sequences were predicted from retrieved genomic or Expressed Sequence Tag (EST) sequences by using consensus splice donor and acceptor site and by sequence identity comparison with related  $Tsh\beta$  or Tshr genes (Table S1 and Table S2).

The signal peptide cleavage site was determined using SignalP (http://www.cbs.dtu.dk/services/SignalP/). Receptor transmembrane domains were predicted using TOPCONS (http://topcons. cbr.su.se/).

### Phylogenetic and syntenic analyses

Alignments were fitted manually using Se-AL editor (http:// tree.bio.ed.ac.uk/software/seal/). The phylogenetic reconstructions were performed on-line by using a maximum likelihood method with PhyMyL 3.0 software [31] on the website file (http:// www.phylogeny.fr/) with HKY85 as substitution model for TSH $\beta$ -related nucleotide sequences and WAG for TSHR-related amino-acid sequences and default settings for the other parameters. The robustness of the reconstruction was estimated by the aLRT score and/or by bootstrapping over 500 replicates. Nucleotide sequences of the entire coding region (including signal peptide) were used for  $Tsh\beta$  subunits with truncation in the 3' end of the longest sequences. The amino acid sequences were used for the TSHR tree reconstruction.

Mapping the genomic neighborhoods of  $Tsh\beta$  and Tshr genes were performed with region overview on Ensembl, NCBI and EBI genome browsers and for the Elephant shark genome on the specific Ensembl website (http://ensembl.fugu-sg.org/index.html).

Flanking genes of duplicated  $Tsh\beta$  and Tshr were identified and annotated in the eel from the eel genome databases, using CLC BIO software.

#### Tissue distribution of $Tsh\beta$ and Tshr transcripts in the eel

Tissue distribution analysis was performed on RNA samples previously prepared from female silver migrating eels caught in the River Loire, France [32]. Total RNA extracted from pituitary, thyroid follicles, olfactory bulb, mesencephalon and diencephalon, telencephalon, cerebellum, medulla oblongata, eyes, liver, intestine, muscle, adipose tissue, gills, and ovary were used. Reverse transcription was performed as previously described [32].

Primers for quantitative real-time PCR (qPCR) for European eel  $Tsh\beta$  (Table S3) were previously reported [32]. Eel specific primer sets for  $Tsh\beta3$ , Tshra and Tshrb were designed using Primer3 [33,34] spanning intron sequences. The specificity of the primer sets was controlled by sequencing PCR product. Moreover, in each case, we checked that the isolated cDNAs of one of the duplicated genes could not be amplified by the primer set corresponding to the other duplicated gene.

Messenger RNA was quantified on LightCycler using the LightCycler FastStart Master plus Sybr green I kit (Roche, Mannhein, Germany) as recommended by the manufacturer. The final primer concentration used was 500 nM. Each sample was run in duplicate using a 1/5 cDNA dilution. The PCR conditions were 95°C for 10 min followed by 50 cycles at 95°C for 5 sec, 60°C for 10 sec and 72°C for 10 sec. The specificity of amplified product was checked by melting curve analysis after the amplification reactions. Relative transcript abundance was calculated from standard curves prepared from pituitaries and thyroid follicles cDNA using LightCycler software. Transcript levels were normalized using total tissue RNA content as previously described in [35].

### **Results and Discussion**

### A 2R-generated $Tsh\beta$ subunit related gene conserved in cartilaginous and in basal lobe-finned fish

Two related  $Tsh\beta$  subunit genes were characterized from the coelacanth genome (Fig. 1A). One of them was in the same genomic region as the known "classical" vertebrate  $Tsh\beta$  subunit



SLC5A8I SYCP1 TSHB TSPAN2-1 NGF KCNA10-1 Spotted gar LG3 29.5 29.5 29.4 29.4 29.3 29.1 Eel sc175 sc3625 0.09/0.03 0.01 sc3025 0.06 0.05 0.08 Zebrafish chr6 49.0 49.0 49.0 49.1 49.1 49.4 Medaka Chr5 11.9 11.9 12.0 11.8 11.9 11.8 Stickleback Gr17 3.48 3.50 3.51 3.50 3.49 3.42 3R Pufferfish Chr11 8.59 8.57 8.56 8.58 8.59 8.64 LGR6 ETV7 TSHB3 TSPAN2-2 NTF7 KCNA10-2 Eel sc3720 -8 sc6543 sc388 Zebrafish Chr23 Ξ 5.0 5.25 0.42 0.45 5.19 5.14 Medaka Chr7 13.7 13.8 13.9 13.7 13.7 Stickleback Gr12 ╋ 12.3 12.3 12.3 12.2 12.2 12.2 Pufferfish Chr9 4.70 4.80 4.79 4.71 4.72

**Figure 1. Syntenic analysis of** *TSH//* **related gene regions.** Genomic regions of  $Tsh\beta$  vs  $Tsh\beta2$  genes (Panel A) and  $Tsh\beta$  vs  $Tsh\beta3$  genes (Panel B) were analysed in representative species (chromosome number or linkage group references are attached to the species name) by using the region overview on the Ensembl genome browser or by blast analysis on the eel draft genome (see Fig. S2A for details). The phylogenetic relationships

А

В

between the representative species are summarized on the right panel. The 3R symbolizes the teleost-specific genome duplication. Genes are named according to the Ensembl nomenclature (Table S4). Gene positions are given (in Mega base) below the symbolized genes. doi:10.1371/journal.pone.0111361.g001

gene. A second gene was located on a genomic fragment that encodes several genes among which three (Mycbpc1, Spic and Ano4) were demonstrated to belong to the fourth paralogous group of genes derived from the duplicated ancGph $\beta$  genomic region (see additional file 9 in [14]). This fourth glycoprotein hormone  $\beta$  subunit gene then clearly represents the Tsh $\beta$  sister gene derived from the 2R and was named Tsh $\beta$ 2.

The genes for the two  $Tsh\beta$  subunit cDNAs (HQ174785 and HQ174784) previously characterized in elephant shark from a pituitary library [14] were present and complete in the new version of the genome, on scaffold\_89 and scaffold\_39, respectively. The former was flanked by genes belonging to the "classical"  $Tsh\beta$  subunit paralogous gene set (Fig. 1A). This was the one unfortunately named  $Tsh\beta2$  when first characterized [14]. The other  $Tsh\beta$  subunit gene on scaffold\_39 was co-syntenic with genes located in the same genomic region as the coelacanth  $Tsh\beta2$  (Fig. 1A). The hypothesis formulated at the time that one of these genes may be the  $Tsh\beta$  sister gene issued from the 2R is thus confirmed.

The skate genome is not fully assembled yet and most of the genes of interest were fragmented into as many contigs as coding exons. The first and second exons of the  $Tsh\beta$  subunit related gene were identified and tentatively linked (Fig. 2).

Only one  $Tsh\beta$  subunit gene was found in the spotted gar genome. It was located on the same genomic region as the classical  $Tsh\beta$  subunit gene (Fig. 1A).

### A 3R-generated $Tsh\beta$ subunit-related gene conserved in most teleosts

The classical  $Tsh\beta$  and a second  $Tsh\beta$  subunit in teleosts were confirmed in a number of representatives from basal elopomorphs like the eel to the acanthomorphs (stickleback, tilapia, tuna, sablefish) through ostariophysian species (Mexican tetra, zebrafish) (Fig. 2, Fig. S1 and Table S1). The first exon of a second  $Tsh\beta$  was also identified in the Atlantic salmon (*Salmo salar*) (Fig. S1, Table S1) suggesting that salmonids also have this second form of TSH. This was not the case in tetraodon and fugu species where only the classical and already characterized  $Tsh\beta$  subunit genes could be found in the complete genome. Synteny analysis shows that the additional  $Tsh\beta$  subunit is located in a conserved genomic region (Fig. 1B), close to the 3R issued duplicated form of Ngf, Ntf7 [36]. This additional Tsh $\beta$  gene was named  $Tsh\beta3$  with reference to the 3R.

## $Tsh\beta$ subunit sequences part into three monophyletic groups

 $Tsh\beta$  subunit sequences of vertebrate representatives were aligned for a phylogenetic analysis. As expected from the synteny analysis, a monophyletic group emerged that clusters the coelacanth  $Tsh\beta2$  together with the elephant shark  $Tsh\beta$ -related subunit HQ174784 (Fig. 3), the protein deduced from the assembled  $Tsh\beta$  exons from the skate and the known  $Tsh\beta$  from the Australian lungfish [37]. The robustness of the monophyletic group that constitutes a sister group to all other  $Tsh\beta$  subunits was strongly supported by a bootstrap value of 93% in 500 replicates. These  $Tsh\beta$  genes were named  $Tsh\beta2$ . The other coelacanth and elephant shark  $Tsh\beta$  genes were included into the "classical"  $Tsh\beta$ cluster at positions compatible with their phylogenetic relationships Although two  $Tsh\beta$  related genes were identified in elephant shark, only one was tentatively characterized in the skate. It branches out with the coelacanth  $Tsh\beta2$ . The classical  $Tsh\beta$ subunit sequence was not found. One possibility is that only one  $Tsh\beta$  subunit was conserved in skate or in holocephals. More likely, however, since Ngf was also absent from the genomic data, the entire locus may have been missed in the sequencing process.

Teleost  $Tsh\beta$  sequences were divided into two monophyletic groups. This is in agreement with the syntenic analysis and supports the hypothesis that they result from the third genomic duplication (3R) that took place early in the radiation of teleosts. The  $Tsh\beta3$  sequence branch length from this phylogenetic tree was 1.6 longer in average (Fig. S4) than for the classical  $Tsh\beta$ sequences indicating that they evolved more rapidly.

The spotted gar belongs to a group that emerged before the radiation of teleosts and its specific genome duplication. It logically lacks the  $Tsh\beta\beta$  gene. It also lacks the  $Tsh\beta\beta$  gene although the genomic region, where it should be located, is well conserved (Fig. 1A) discarding a possible problem with the sequencing data. It then seems that the  $Tsh\beta\beta$  gene was lost at least twice, in the lobe-finned fish lineage before the radiation of tetrapods and early in the ray-finned fish lineage. It must be of significance that teleosts specifically retained the newly generated  $Tsh\beta\beta$  gene when they lost the other 3R-generated  $Gph\beta$  gene duplicates [38].

### TSH $\beta$ 2 and TSH $\beta$ 3 sequences present specific signatures

Both TSHB2 and TSHB3 retained most structural features shared by all types of glycoprotein hormone  $\beta$  subunits like the cysteine residues and many other amino acids that are conserved in position (Fig. 2) indicating that these sequences are subject to functional constraints. It is then most likely that they are able to associate to an  $\alpha$  subunit and form an active heterodimer. TSH $\beta$ 2 and TSH $\beta$ 3 are predicted to be cleaved from the signal peptide at roughly the same position as in the classical TSH $\beta$ , one or two amino acids before the first conserved cysteine residue (Fig. 2). Sequence alignment indicates that five amino acid positions are well conserved in the TSH $\beta$ 2 group that are different to or variable in the classical TSH $\beta$  subunit sequences. Two are located within the first exon and 3 within the second. The amino acid composition from the two associated skate exonic sequences are well in agreement with their assembly into a unique gene belonging to the  $Tsh\beta 2$  group. TSH $\beta 2$  and TSH $\beta$  subunits share the two additional amino acid residues between the cysteines 5 (the last encoded by the 1<sup>st</sup> exon) and 6 (20 amino acids apart) as compared to LH $\beta$  and FSH $\beta$  subunits [39]. By comparison, in the tunicate ciona GPB5, like in the gonadotropin  $\beta$  subunits, the homologous cysteine residues are 18 amino acids apart [40,41]. Given tunicates are the closest relatives to vertebrates [42] and since  $ancGph\beta$  was generated by a duplication of Gpb5 just prior to the emergence of vertebrates [14], it is likely in the ancGPH $\beta$ subunit precursor, the cysteines were also 18 amino acids apart. Thus, the *preTsh* $\beta$  evolutionary precursor likely acquired these two codon insertions at the time of the 1R. It can be inferred from the aligned sequences that the insertion/deletion event was not generated at the splice site (Fig. 2). In the coelacanth  $TSH\beta$ sequences however, the splice site is shifted twelve nucleotides towards the 3' end.

TSH $\beta$ 3 sequences display particular signatures (Fig. 2 and Fig. S1). The most significant is that TSH $\beta$ 3-type subunits harbor two potential N-linked glycosylation sites. The glycosylation pattern of

FSHβ       European eel      HILAVTALCITLAPILARASTSCIANTISTSVENECCGCVT         TSHβ3       Siblefin      MCLPVEKCHLCALMERAVCACHLNHTIWTERBCAQCVA         TSHβ3       Siblefin tuna      MCLPVEKCHLCALTSTVQACHLNHTIWTERBCAQCVA         TSHβ3       Siblefin tuna      MCLPVEKCHLCALTSTVQACHLNHTIWTERBCAQCVA         TSHβ3       Medaka      MELVLKCHLCALTARAGCACHSHTTWTERBCAQCVA         TSHβ3       Atlantic cod       MDRVTLSSNSLFVCVLLCVLLQLAGALACVPQWTLYVEKPECGPCVA         TSHβ       Zebrafish      MRULLGSFLLLGEDALAGCVPQWTLYVEKPECGPCVA         TSHβ       Zebrafish      MRUVLLASAVLCLLAGQVLSICSPVDYTLYVEKPECDFCVA         TSHβ       Zebrafish	
TSHβ3       Sablefish        MPLLUKCTLLCALMERAVCACMLKNHTIWIERBCAQCVA         TSHβ3       Slickleback         TSHβ3       Slickleback         TSHβ3       Slickleback         TSHβ3       Atlantic cod         MDRVYLSSMSLFVCVLLCVLIADTACGCTLKNFTLMIEKVECQCVL         TSHβ3       Zebrafish        MRULCSFLLLGEDALLACSLKNTTLVVEKVECQCVL         TSHβ       Zebrafish        MRVULLASAUCLLAQULSICSPVDYTLYVEKVECQCVL         TSHβ       European eel        MRVULLASAUCLLAQULSICSPVDYTLYVEKVECPCDYCVA         TSHβ       Atlantic cod         TSHβ       Atlantic cod         TSHβ       Atlantic cod         TSHβ       Medaka        METAVPPCWLLFLLSPAVPTCFPTDFTMYVERPECPCYCVA         TSHβ       Slickleback        METAVPFCWLLFLLSPAVPMCFPTDFTLYVERPECPCYCVA         TSHβ       Sluegen        METAVFTCWLLFLSPAVPMCFPTDFTLYVERPECPCYCVA         TSHβ       Sluegen        METAVFPCWLLFLSPAVPMCFPTDFTLYVERPECOFCVA         TSHβ       Sluegen	F <mark>NTTACAGLC</mark> FTQ <mark>D</mark> SVYKSSLKPYP <b>Q</b> QACNF
TSHβ3       Bluefin tuna        MCLFVFKCHLLCALHSGTVCACHLKNHTIWTERDCACCVA         TSHβ3       Midaka        MEVLLKCMLLCALMHRAACACMLSNHTIWTERDCACCVACUL         TSHβ3       Midaka         TSHβ3       Midaka         TSHβ3       Midaka         TSHβ3       Atlantic cod         MRVVLSSNSLFVCVLLCVLLAGACKVNTTIWTERSCECOCUL         TSHβ       European eel        MRVULSSNLLAGQULSTSPVDTTIVTERSECDFCVA         TSHβ       Atlantic cod         TSHβ       Medaka        MRVVLLSAVULLAGQULSTSPVDTTIVTERSECDFCVA         TSHβ       Bildefin tuna        MRTAVPPCWLLFLLSPAVPTCFPTDTTMVVERFECDFCVA         TSHβ       Sickleback        MTTAMFTCWLLFLLSPAVPTCFPTDTTMVVERFECDFCVA         TSHβ       Sickleback        MTTAMFTCWLLFLLSPAVPMCFPTDTTLVVERFECDFCVA         TSHβ       Sickleback        MTAMPTCWLLFLLSPAVPMCFPTDTTLVVERFECDFCVA         TSHβ       Sickleback        MTAMPTCWLLFLLSPAVPMCFPTDTTLVVERFECDFCVA         TSHβ       Sickleback        MTAMPTCWLLFLLSPAVPMCFPTDTTLVERFECDFCVA         TSHβ       Sickleback        MTAMPTCWLLFLLSPAVPMCFPTDTLVERFECDFCVA         TSHβ <td< th=""><th>I<mark>NTTICNGYC</mark>YTQ<mark>DT</mark>NLRGRFGRSFVI<b>Q</b>RSCVF</th></td<>	I <mark>NTTICNGYC</mark> YTQ <mark>DT</mark> NLRGRFGRSFVI <b>Q</b> RSCVF
TSHβ3       Stickleback         TSHβ3       Mtantic cod         MDRVTLSSNSEPCVLLCVLLADTACGCTLKNFTIMTEKVECQCVL         TSHβ3       Atlantic cod         TSHβ3       European eel         TSHβ4       Mtattic cod         TSHβ3       Atlantic cod         TSHβ4       European eel         TSHβ4       Atlantic cod         TSHβ4       Mtattic cod         TSHβ5       Stickleback	I <mark>NTTICSGYC</mark> YTQ <mark>D</mark> TNLKGWFGRTFLI <b>Q</b> RSCVF
TSHβ3       Medaka      MSLFMLKSALVLAVMAGTVCACULKNFTIMIEKVECQCVL         TSHβ3       Zebrafish      MRVLLCSFLLLGEDALLACSLKNYTLYVEKBECGECHCVA         TSHβ3       European eel      MRVLLCSFLLLGEDALLACSLKNYTLYVEKBECGECHCVA         TSHβ       European eel      MRVLLSSAVLCLLAGQUISICSPVDTILYVEKBECGECHCVA         TSHβ       Atlantic cod      MRVULLASQVLLLAGQUISICSPVDTILYVEKBECDFCVA         TSHβ       Atlantic cod      MSLVVIGMLGLLMKVAPMCAPPDTPTDTIVTERGECNYCVA         TSHβ       Atlantic cod      MSLVVIGULLLLSPAPPCCPTPDTTMYVEREPECDYCVA         TSHβ       Skikeback      METAVPFCULLFLLSPAPPCCPTPDTPTMYVEREPECDYCVA         TSHβ       Skikeback      METAVPTCULLFLLSPAPPMCPTPDTFTMYVEREPECDYCVA         TSHβ       Skikeback      METAVPTCULLFLLSPAPPMCPTPDTFTMYVEREPECDYCVA         TSHβ       Skikeback	I <mark>NTTICKGYC</mark> YTK <mark>D</mark> TNLKGRFGRDFMIQRSCVF
TSHβ3 Atlantic cod       MDRVYLSSMSLFVCVLLCVLIADTAGCCILKPTLINEXVECCQCUL         TSHβ3 Zebrafish      MRVLLCSPLLLGQALACCYPNYTLYVEKHECGHCMA         TSHβ European eel      MRUVLLASAVLCLLAQQLASCVPNYTLYVEKHECGHCMA         TSHβ Atlantic cod      MSULYVIGMLGLUKKVAVPACAPTDYTLYVEKHECGHCVA         TSHβ Atlantic cod      MSULYVIGMLGLUKKVAVPACAPTDYTLYVEKPECDYCVA         TSHβ Atlantic cod      MDYFVFVGSVLLMFSPAAPACVPTDYTLYVEKPECDYCVA         TSHβ Bitalnic cod      MDYFVFVGSVLLMFSPAAPACVPTDYTLYVEKPECDYCVA         TSHβ Bitalnic cod      MDYFVFVGSVLLMFSPAAPACVPTDYTLYVEKPECDYCVA         TSHβ Solted gar      MTDAWFCCULFLLSPAVPTCPTDFTTVVEKPECDYCVA         TSHβ Solted gar      MSAAUTCCLLCUASQTLSKCAPTDYMLVEKRECAYCVA         TSHβ Solted gar      MSAAUTCCLLCUASQTLSKCAPTDYMLVEKRECAYCVA         TSHβ Solted gar      MSAAUTCCLLCUASQTLSKCAPTDYMLVEKRECAYCVA         TSHβ Solted gar      MSAAUTCCLLCUASQTLSKCAPTDYMLVEKRECAYCVA         TSHβ Solted gar      MSAAUTCCLLCUASQTLSKCAPTDYMLVEKRECAYCUA         TSHβ Solted gar      MSAAUTCCLLCUASQTLSKCAPTDYMLVEKRECAYCUA         TSHβ Solted gar      MSAAUTCCLLCUASQTLSKCAPTMYLVEKRECAYCUA         TSHβ Solted gar      MSAAUTCCLLCUASQTLSKCAPTDYMLVEKRECSCLWCA         TSHβ Solted gar      MSAAUTCCLLVASQLSCISCLTBRVVVEKRECSCLWCUASKA         TSHβ	I <mark>NTTICSGYC</mark> YSRD <mark>T</mark> NFRGRFGRTFLI <b>Q</b> RS <b>C</b> MF
TSHβ3       Zebrafish         TSHβ3       European eel         TSHβ4       Zebrafish         TSHβ4       Zebrafish         TSHβ4       Zebrafish         TSHβ4       Zebrafish         TSHβ4       Allantic cod         TSHβ4       Malantic cod         TSHβ4       Medaka         TSHβ5       Medaka         TSHβ5       Stickleback         TSHβ5	I <mark>NTTICSGYC</mark> YTQ <mark>D</mark> TNFRGRVGKNFLI <b>Q</b> RG <b>C</b> TF
TSHβ3       European eel      MLDSLACVLLCLLLQQALAKCVPQNYTLYVERPECCPCVA         TSHβ       European eel      MSLVULLASAVLCLLAQVLSICSPUDYTLYVERPECDFCVA         TSHβ       Atlantic cod      MSLVUCMAGLMKVAVPMCAPTDYTTIVERPECDFCVA         TSHβ       Atlantic cod      MSLAVUCMAGLMKVAVPMCAPTDYTTIVERPECDYCVA         TSHβ       Medaka      METAVPPCMLLFLLSPAVPTCPTDFTMYVERPECDYCVA         TSHβ       Bildefin tuna      METAVPTCMLLFLLSPAVPTCPTDFTMYVERPECDYCVA         TSHβ       Sablefish      MSTAVFTCMLLFLFSPAVPMCPTDFTMYVERPECDYCVA         TSHβ       Sablefish      MSTAVFTCMLLFLLSPAVPMCPTDFTMYVERPECDYCVA         TSHβ       Sablefish      MSTAVFTCMLLFLFSPAVPMCPTDFTMYVERPECDYCVA         TSHβ       Sablefish      MSTAVFTCMLLFLSPAVPMCPTDFTMYVERPECDYCVA         TSHβ       Coelacanth       /YYLYRMNHICLVSILLYLVRQALSICSLTQHTMYVERECCSCMA         TSHβ2       Little shate      MSCMWLSLLLILFLCGGRAPHYCSPSYLQUEQDOCCFCLV         TSHβ2       Little shate      MNCMWLSLAVLUCKSCSUNSLCTVTRYMVVEREECSHCMA         TSHβ2       Little shate      MNCWLLLAVLUCKSCSUNSLCTVTRYMVVEREECSHCMA         TSHβ2       Little shate      MNCWLULLAVLUCKSCSUNSLCTVTRYMVVEREECSHCMA         TSHβ2       Little shate	I <mark>NTTVCSGMC</mark> FTRD <mark>T</mark> NVQGFVGKRFLL <b>Q</b> QS <b>C</b> MF
TSHβEuropean eelMRVVLLASAVLCLLAGQVLSICSPVDYTLYVERPECDFCVATSHβAtlantic codMSLTVVIGMLGLLMKVAPPACAPTDYTLVERPECDYCVATSHβMalantic codMDYFVFVGSVLLMFSPAAPmCVPTDYTLVVERPECDYCVATSHβMedakaMTAVFPCKLLFLLSPAVPTCFPTDFTMVVERPECDYCVATSHβSticklebackMTATAVPPCKLLFLLSPAVPTCFPTDFTMVVERPECDYCVATSHβSablefishMTATAVPCKLLFLLFSPAVPMCLPTDFTLVVERPECDYCVATSHβSablefishMGAALLVCCLLCLVASQTLSKCAPTDYMLVERVGCAYCVATSHβSobted garMSAAVLTCALLCLMAGNASSLCEPTAYTLVVERVGCAYCVATSHβSobted garMSAAVLTCALLCLMAGNASSLCEPTAYTLVVERVGCAYCVATSHβSobted garMSAAVLTCALLCLMAGNASSLCEPTAYTLVVERVGCAYCVATSHβSobted gar	V <mark>NTTVCRGFC</mark> FSRD <mark>TN</mark> MKK <mark>C</mark> GLKGFPVQRACMY
TSHβ       Zebrafish         TSHβ       Atlantic cod         TSHβ       Medaka         TSHβ       Medaka         TSHβ       Stickleback         TSHβ2       Lingtrickleback         TSHβ2       Stickleback         TSHβ3       Stickleback         TSHβ3       Stickleback	I <mark>NTTICMGFC</mark> YSLD <mark>PN</mark> VVGPAVKRLVVQRGCTY
TSHβ       Atlantic cod      MDYFVFVGSVLLLMFSPAAPMCVPTDYTLYVEKPECNFCVA         TSHβ       Medaka      METAVFPCWLLFLLSPAVPTCFPTDFTMYVERPECDYCVA         TSHβ       Stickleback      METAVFPCWLLFLLSPAVPTCFPTDFTMYVERPECDYCVA         TSHβ       Bluefin tuna      METAVFTCWLLFLLFSPAVPMCLPTDFTLYVEKPECDYCVA         TSHβ       Sablefish      METAVFTCWLLFLLFSPAVPMCLPTDFTLYVEKPECDYCVA         TSHβ       Soblefish      METAVFTCWLLFLLFSPAVPMCLPTDFTLYVEKPECDYCVA         TSHβ       Soblefish      METAVFTCWLLFLLFSPAVPMCLPTDFTLYVEKPECDYCVA         TSHβ       Soblefish      METAVFTCWLLFLLSSALCEPTAYTLYVEKPECDYCVA         TSHβ       Soblefish      METAVFTCWLLFLLSSALCEPTAYTLYVEKPECSYCVA         TSHβ       Coelacanth       /YULYRNNHICLVSILLYLLSSALARAGCSTRHVLYVEKEGCNFCMA         TSHβ2       Lingfish      MNCLWLLPAVLLLGSSQIGFTCSTRHVVYEKEGCNFCMA         TSHβ2       Longfish      MNCLWLLPAVLLLGSCSUCTMSRYMLYIERECSHCMA         TSHβ2       Soblefish       SUVRCHULPCCPGNNSQLYPPAAGCSCTRRHVLYVEKEGCNFCMA         TSHβ3       Sablefish       HSUVYETVLLFCCCHLVSAGGSLLPCEPINETINECVFRKL         TSHβ3       Sablefish       SUVRANHVPGCPQONNSQLYPPAAGCSCRRCDTRTHCVTRSR-         TSHβ3       Stickleback       LSUVRANHVPGCPQNNPQUYPAAGCSCRCCDRCTRTHCVTRSR-         TSHβ3 <th>V<mark>NTTICMGFC</mark>FSR<mark>D</mark>SNIKELVGPRFIV<b>Q</b>RG<mark>C</mark>TY</th>	V <mark>NTTICMGFC</mark> FSR <mark>D</mark> SNIKELVGPRFIV <b>Q</b> RG <mark>C</mark> TY
TSHβ       Medaka      METAVFPCWLLFLLSPAVPTCFPTDFTMYURPPECDYCVA         TSHβ       Stickleback      METAVFPCWLLFLLSPAVPTCFPTDFTMYURPPECDYCVA         TSHβ       Sablefish      METAVFTCWLLFLLSPAVPMCFPTDFTMYURPPECDYCVA         TSHβ       Sablefish      METAVFTCWLLFLLSPAVPMCFPTDFTMYURPPECDYCVA         TSHβ       Sablefish      METAVFTCWLLFLLSPAVPMCFPTDFTMYURPPECDYCVA         TSHβ       Sobled gar      METAVFTCWLLFLLSPAVPMCFPTDFTMYURPPECDYCVA         TSHβ       Sobled gar      METAVFTCWLLFLLSPAVPMCFPTDFTMYURPPECDYCVA         TSHβ       Sobled gar      METAVFTCWLLFLLSAVPTCPTDFTMYURPPECDYCVA         TSHβ       Sobled gar      METAVFTCWLLFLLSAVATUVESACAPTDYMLYURPECDYCVA         TSHβ       Selecanth       /YULYINNHICLCUSILLYUVRQALSICSLTAPHTIYURPPECAUCAVCA         TSHβ       Selecanth      MNCCSMRVLSLLPLLLSAAARAQCSLTRHVUYUREECSUCMA         TSHβ2       Coelacanth      MNCCSMRVLSLLPLLLSAAARAQCSLTRHVUYUREECSUCMA         TSHβ3       Sablefish      MNCCSMRVLSLLPLLLSAAARAQCSLTRHVUYUREECSUCMA         TSHβ2       Coelacanth      MNCCSMRVLPVUICMSCTSVNSLCTVRYMMYUREECSUCMA         TSHβ3       Sablefish       HSLVYRAAHVPGCPQDVNSQLYPPALSCCSCRCDTRTHHCVRTR-         TSHβ3       Sablefish       HSLVYRAAHVPGCPQNNSQLYPPAARCCKRCDTRTHHCVRTSR-	I <mark>NTTICMGFC</mark> YSR <mark>D</mark> SNIGDLVGLRFLL <mark>Q</mark> RG <mark>C</mark> TY
TSHβSticklebackMETAVFPCWLLFLLLSPAVPTCFPTDFTMYVERPECDYCVATSHβBluefin tunaMETAVFTCWLLFLLFSPAVPMCLPTDFTLYVERPECDYCVATSHβSablefishMETAVFTCWLLFLLFSPAVPMCLPTDFTLYVERPECDYCVATSHβSpotted garMETAVFTCWLLFLLFSPAVPMCLPTDFTLYVERPECDYCVATSHβSturgeonMETAVLTCALLCLANGNASSLCEPTAYTLYVERPECAYCVATSHβSturgeonMETAVLTCALLCLANGNASSLCEPTAYTLYVERPECAYCVATSHβCoelacanth/YYLYRNNHICLVSTLLYLVVRQALSICSLTQHTIYVEKEECSYCMATSHβ2Elephant sharkMETAVLPLLEGGRAHPYCSPSPYLQYLEQDQCEFCLVTSHβ2Little skateMCCSMRVLSLLPLLLSLAAARAQCSLTRHVLYVEKEECSYCMATSHβ2LongfishMNCLWLPAVLLHCRPVGSLCTMSRWLLTEREECSHCMATSHβ2LooelacanthMNTWLVPVVICMSCTSVNSLCTVTRYMMYVEKEQCSHCIALHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLLPCPTINETISVEKDGCPKCLVTSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYPPAALSCECSKRCDSTHCVRSSQ-TSHβ3StoklebackLSLVYRAAHVPGCPQNNVPQLYPPAALSCECSKCNDSTDCGPLNTTTSHβ3SablefishHSLVYRAAHVPGCPPUNPLYSPVALGCCKCRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRAVHPGCPPUNPLYSPVALGCCCRCCCCCCCCNSSQ-TSHβ3StoklebackLSLVYRAVHPGCPPUNPLFSPVALGCCCCCCCCCCCNSDECCHRASGTSHβ3European eelQSLVYHAVSLPGCPPUNPLFSPVALSCHCCSCCCTCNTSDECCHRASGTSHβ3European eelQAVEYRTALLPGCPTDSDPYSPVALSCHCCACNTAVDECHRASGTSHβ3European eelQSLVYHAVSLPGCPPHUDPHFSPVALSCHCCACNTANDECHRASGTSHβ4Atlantic codNQEYRTAILPGCPTDSDPYSPVALSCHCCACNTANDECHRASGTS	I <mark>NTTICMGFC</mark> YSR <mark>D</mark> SNVRAIVGPRFLIQTG <mark>C</mark> NY
TSHβ       Bluefin tuna      MTDTAMFTCWLLFLLFSPAVPMCLPTDFTLYVEKPECDFCVA         TSHβ       Sablefish      METAVFTCWLLFLLFSPAVPMCPPTDFTLYVERPECDYCVA         TSHβ       Spotted gar      MSAAULTCALLCLAMGNASSLCEPTATLYVERPECDYCVA         TSHβ       Sturgeon      MSAAULTCALLCLAMGNASSLCEPTATLYVERPECDYCVA         TSHβ       Coelacanth       /YYLYRMNHICLVSILLYLVVRQALSICSLTOHTIYVEKECCYCVA         TSHβ2       Elephant shark      MPFCRMSSRLLLILFLCGGRAHPYCSPSYLQYLEQDQCEFCLV         TSHβ2       Little skate      MNAMULPLVLCLSGSQIGFTCSLTRHVYVEKECCYCMA         TSHβ2       Little skate      MNCSMRVLSLPLLLSLAARAQCSLTRHVYVEKECCSHCMA         TSHβ2       Longfish      MNFTWLVPVVICMSCTSVNSLCTVTRYMMYVEKEQCSHCIA         LHβ       European eel       /YPECTWLLPGCPQONNGLYTPAARCSCCKCDTATHHCVTRR-         SHβ3       Sablefish       HSLVYRAHPGCPPOVNSQLYTPAARCSCCRCDTATHHCVTRSQ-         TSHβ3       Sluefin tuna       LSLVYRAVHPGCPPOVNPQUYPAAHCCKRCDRTHHCVRTSSQ-         TSHβ3       Medaka       LSUVYRAVHPGCPPOVNPQUYPAAHCCKRCDRTHHCVRTSSQ-         TSHβ3       Sluefin tuna       SLVYRAAHPGCPPOVNPQUYPAAHCCKRCTRATSHCVFRHK-         TSHβ3       Medaka       LSUVYRAAHPGCPPOVNPQUYPAAHCCKRCCACTATHHCVQKSSQ-         TSHβ3       Luefn tuna       SLVYRSARMPGCPVHIDPLFFYPVALSCHCSRCMTHHCVQKSSA-	I <mark>NTTICMGFC</mark> YSR <mark>D</mark> SNVRAIVGPRFLIQTG <mark>C</mark> NY
TSHβ       Sablefish         TSHβ       Spotted gar         TSHβ       Storgeon         TSHβ       Sturgeon         TSHβ       Coelacanth         YYLRMNHICLVSILLYLVVRQLSICSTPOYMLVVEKYCCAYCVA         TSHβ       Coelacanth         YYLRMNHICLVSILLYLVVRQLSICSTPOYMLVVEKYCCAYCVA         TSHβ       Coelacanth         YYLRMNHICLVSILLYLVVRQLSICSTPOYMLYVEKYCCAYCVA         TSHβ2       Elephant shark        MCCSMRVLSILPLULSLAAARAQCSITRHUTVVEKECCSYCMA         TSHβ2       Coelacanth         TSHβ2       Coelacanth         TSHβ2       Coelacanth        MCCSMRVLSILPLLLSLAAARAQCSICTMSRYMLYIERECSYCMA         TSHβ2       Coelacanth         TSHβ2       Coelacanth         TSHβ2       Coelacanth        MCCSMRVLSILPLLLSLAAARAQCSICTMSRYMLYIERECSHCMA         TSHβ3       Storgeonth         SHβ4       European eel         RDVVYETVHLPGCPQONNSQLYPAAQCSCSCRCDTRTHHCVRTSQ-         TSHβ3       Storgeonth         Storgeonth       IsuvrAAHPGCPQONNPQUYFPAAHRCLCKRCDTRTHHCVRTSQ-         TSHβ3       Storgeonth         Storgeonth       IsuvrAAHPGCPQNNVPUYFPAAHRCLCKRCDTRTHHCVRTSQ-         TSHβ3       Storgeonth	I <mark>NTTICMGFC</mark> YSR <mark>D</mark> SNMRDILGPRFLIQKGCTY
TSHβSpotted garMGAALLVCGLLCLVASQTLSKCAPTDYNLYVEKYGCAYCVATSHβSturgeonMSAAVLTCALLCLANGNASSLCEPTAYTLYVERQECAYCVATSHβCoelacanth/YYLYRMNHICLVSILLYLVVRQALSICSLTQHTIYVEKRECTSCLVTSHβElephant sharkMCGMSRULLILFLCGGRAHPYCSPSPYLQYLEQDQCEFCLVTSHβ2Elephant sharkMCASRVLSLLPLLSLAAARAQCSLTRHVUYVEKECCYCMATSHβ2Little skateMCCSMRVLSLLPLLSLAAARAQCSLTRHVUYVEKECCYCMATSHβ2LungfishMNCTULLPAVLLHCPVGSLCTMSRYMLYIEREECSHCMATSHβ2CoelacanthMNFTWLVPVVICMSCTSVNSLCTVTRYMMYVEKEQCSHCIALHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLPCEPINETISVEKDGCPKCLVFSHβSablefishHSLVYRAHVPGCPQOVNSQLYYPAAQRCSCRCCDTRTHHCVRTRR-TSHβ3SloklebackLSLVYRAVHPGCPPOVNSQLYYPAAQRCSCRCCDTRTHHCVRTSR-TSHβ3StoklebackLSLVYRAVHPGCPPOVNALYPAHCCSCRCCTRTHHCVRTSR-TSHβ3Atlantic codGSLVYTARLVGCPRNVNPVIYYPEHRCKCRSCDRTHHCVQFRR-TSHβ3European eelQAVEYTARLPGCPPHVDPFFYVARCCCTRCTRTHHCVRTSR-TSHβ3European eelQAVEXTARLPGCPHUDPLFFYPVARCCCRCCTRTHHCVRSR-TSHβ3MedakaLSLVYRAVLPGCPPHVDPFSYPVALSCHCGRCTATADECAHRAS-SHβ3European eelQAVEXTAELPGCPHVDPRFSYPVALSCHCGCRCTDSDECHRRS-SHβ3European eelQAVEXTAELPGCPHDPFFYPVALSCHCGACRTDSDECAHRRS-SHβ3European eelQAVEXTAELPGCPHDPFYPVALSCHCGACRTDSDECAHRAS-SHβ4European eelQAVEXTAELPGCPHDPFYPVALSCHCGACRTDSDECAHRAS-SHβ5DKVEXTAELPGCPHDDPFYPVALSCHCGACRTDSDECAHRAS-SHβ4Bluefin tunaDKVEX	I <mark>NTTICMGFC</mark> YSR <mark>D</mark> SNVRAIVGPRFLIQTG <mark>C</mark> TY
TSHβSturgeonMSAAVLTCALLCLAMGNASSLCEPTAYTLYVERQECAYCVATSHβCoelacanth/YYLYRMNHICLVSILLYLVVRQALSICSLTQHTIVVEKRECTSCLVTSHβElephant sharkMCSARVLSILFLITFLCGGRAHPYCSPSPLQVLEQQCEFCLVTSHβ2Elephant sharkMCCSMRVLSLPLLSGSQIGFTCSLTRHVVYVEKRECCSYCMATSHβ2Little skateMCCSMRVLSLPLLLSLAAARAQCSITRHVLYVEKRECCSYCMATSHβ2LungfishMCCSMRVLSLPLLLSLAAARAQCSITRHVLYVEKRECCSHCMATSHβ2CoelacanthMCCSMRVLSLPLLLSLAAARAQCSITRHVLYVEKRECCSHCMATSHβ2CoelacanthMCSARVLSLPLLLSLAAARAQCSITRHVLYVEKRECCSHCMATSHβ2CoelacanthMNTTWLVPVVICMSCTSVNSLCTVTRYMMYVEKRECCSHCLALHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLLPCEPINETISVEKDGCPKCLVTSHβ3SablefishHSLVYRAHVPGCPQDVNSQLYPAAQRCSCRCCDTRTHHCVRTRR-TSHβ3SablefishHSLVYRAHVPGCPQDVNSQLYPAAQRCSCRCCDTRTHHCVRTSR-TSHβ3Bluefin tunaLSLVYRAVHLPGCPPGVNPQVYPAAHRCLCKRCDTRTHHCVRTSR-TSHβ3Atlantic codGSLVYRARLVGCPRNVNPVI YYPEHRCKCRSCDRTHHCVQKSR-TSHβ3Leuropean eelQAVEXTAELPGCPPNDPLFSPVALRCCRCCTCKNTSNTECLHRGK-TSHβZebrafishRSLVYRSARMPGCPVHDPFFSPVALRCCRCCRCTARDECHRAS-TSHβEuropean eelQAVEXTAELPGCPENDPHFSYPVALSCHCGACRTDSDECAHRAS-TSHβZebrafishRSLVYRAALLPGCPIDSDPFSYPVALSCHCGACRTDSDECAHRAS-TSHβEuropean eelQAVEXTAELPGCPENDPFSYPVALSCHCGACRTDSDECAHRAS-TSHβAtlantic codNQVEXTAELPGCPENDPFSYPVALSCHCGACRTDSDECAHRAS-TSHβBluefin tunaDKVEYRAILPGCPENDPFSYPVALSCHCGACRTDSDECAHRAS- <th>I<mark>NTTICSGFC</mark>YSR<b>D</b>TNVKGVVGKSYFL<mark>Q</mark>RSCTY</th>	I <mark>NTTICSGFC</mark> YSR <b>D</b> TNVKGVVGKSYFL <mark>Q</mark> RSCTY
TSHβCoelacanth/YYLYRMNHICLVSILLYLVVRQALSICSLTQHTIYVEKRECTSCLVTSHβElephant sharkMRASSRLLLILFLCGGRAHPYCSPSPYLQYLEQDQCEFCLVTSHβ2Elephant sharkMRASSRLLLILFLCGGRAHPYCSPSPYLQYLEQDQCEFCLVTSHβ2Little skateMRAWLLPLVLCSGSQIGFTCSLTRHVYVEKRECSYCMATSHβ2Little skateMRAWLLPLVLLSGSQIGFTCSLTRHVYVEKRECSCHCMATSHβ2CoelacanthMRCLWLLPAVLLLHCPPVGSLCTMSRYNLYIERRECSHCMATSHβ2CoelacanthMRTWLVPVVICMSCTSVNSLCTVTRYMMYVEKRECSCHCIALHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLPCEPINETISVEKDGCPKCLVTSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYYPAAQRCSCRCCDTRTHHCVRTRR-TSHβ3SablefishHSLVYRAHLPGCPPQVNPQLYFPVALGSCRCCDTRTHHCVRTSR-TSHβ3SticklebackLSLVYRVHLPGCPPDVNAELYYPVAHGCSCRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRAVHLPGCPPDVNAELYPVAHGCSCRCDTRTHHCVRTSR-TSHβ3ZebrafishRSLVYRSARMPGCPVHIDPLFFYPVARCCKCCTCCTCCTSRNECVFRHK-TSHβ3European eelQSLVYHAVSLPGCPPDVDPLFSFPVALKCHCSRCMTSNTECLHRCK-TSHβ3European eelQAVEYRTAELPGCPDNDPLFSFPVALKCHCSRCMTSNTECLHRCK-TSHβ3European eelQAVEYRTAILPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRRS-TSHβ3SticklebackDKVEYRTAILPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRRS-TSHβ4Atlantic codNQVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECVHRRS-TSHβ4SticklebackDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβ4SticklebackDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβ5SolefishDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECA	INTTICAGFCVTRDVNLKSLLPKSALSQSSCTY
TSHβElephant shark-MPMFCRMSSRLLLLILFLCGGRAHPYCSPSPYLQYLEQDQCEFCLVTSHβ2Elephant sharkMRAMWLLPLVLCLSGSQIGFTCSLTRHVVYVEKECSYCMATSHβ2Little skateMRCSMRVLSLEPLLSLAAARAQCSLTRHVLYVEKECSYCMATSHβ2CoelacanthMRTWLVPVVLCMSCTSVNSLCTVTRYMMYVEKEQCSHCIALHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLPCEPINETISVEKDGCPKCLVFSHβ3SablefishHSLVYRAHLPGCPQDVNSQLYPPAAQRCSCRCDTRTHHCVRTRR-TSHβ3SablefishHSLVYRAHPGCPQDVNSQLYPPAAQRCSCRCDTRTHHCVRTRR-TSHβ3SticklebackLSLVYRAVHLPGCPQDVNPQLYPVALGCSCRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRAVHLPGCPPDVNAELYPVAHGCSCRCDTRTHHCVRTSR-TSHβ3ZebrafishRSLVYRSARMPGCPPVVDPLFSFPVALRCKCRSCDRTHHCVRSR-TSHβ3European eelQAVEYRTALPGCPPDVDPLFSFPVALRCKCRSCDRTHHCVRSR-TSHβ3European eelQSLVYHAVSLPGCPPDVDPLFSFPVALRCKCRSCDRTHHCVRSR-TSHβ3European eelQSLVYHAVSLPGCPPDVDPLFSFPVALRCKCRSCDRTHHCVRSR-TSHβEuropean eelQAVEYRTALPGCPSGSSLFSYPVALSCHCGACNTAVDECHRAS-TSHβEuropean eelQVEYRTALPGCPSGSSLFSYPVALSCHCGACRTDDECTLRRAS-TSHβSticklebackDKVEYRALLPGCPIDDPVFSYPVALSCHCGACRTDDECCHRAS-TSHβSticklebackDKVEYRALLPGCPIDSDPVSYPVALSCHCGACRTDDECCHRAS-TSHβSticklebackDKVEYRTALLPGCPINAVFTYPVALSCHCGACRTDDECCHRAS-TSHβSticklebackDKVEYRTALLPGCPINAVFTYPVALSCHCGACRTDDECCHRAS-TSHβSticklebackDKVEYRTALLPGCPINAVFTYPVALSCHCGACRTDDDECCHRAS-TSHβSticklebackDKVEYRTALLPGCPINAVFTYPVALSCHCGACRTDDDECCHRAS-TSHβ </th <th>VNTTICSGYCRTRVIMKNRLLPKTALSOHVCTY</th>	VNTTICSGYCRTRVIMKNRLLPKTALSOHVCTY
TSHβ2Elephant sharkMNAMWLLPLVLCLSGSQIGFTCSLTRHVVYVEKECSYCMATSHβ2Little skateMCCSMRVLSLLPLLLSLAAARAQCSLTRHVLYVEKEGCNFCMATSHβ2LungfishMNCLWLLPAVLLHCREVGSLCTMSRYMLYIEREECSHCMATSHβ2CoelacanthMNFTWLVPVVICMSCTSVNSLCTVTRYMVVEKEQCSHCIALHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLLPCEPINETISVEKDGCPKCLVFSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYPPAADCCSCRRCDTRTHHCVRTRFTSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYPPAADCCSCRRCDTRTHHCVRTSR-TSHβ3SticklebackLSLVYRAVHLPGCPPGVNPQLYFPVALCCSCRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRAVHIPGCPPDVNAELYPVAHCCSCRCDTRTHHCVRTSR-TSHβ3Lurgean eelQSLVYRAVLPGCPPDVNAELYPVAHCCSCRCDTRTHHCVRTSR-TSHβ3European eelQSLVYRAVHIPGCPPDVNAELYPVAHCCSCRCDTRTHHCVRTSR-TSHβ3European eelQSLVYRTALLGCPPNVNPVIYYPEHRCKCRSCDRTHHCVRSSR-TSHβ3European eelQAVEYRTALPGCPPDVDPLFSPVALRCHCSRCNTSRNECVFRHK-TSHβEuropean eelQAVEYRTALPGCPPHVDPRFSYPVALSCHCGSCNTAVDECAHRAS-TSHβKebrafishQEVEYRTALPGCPSEGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβSticklebackDKVEYRAALLPGCP IDSDPVFSYPVALSCHCGACRTDSDECVHRAPGEPTSHβBluefin tunaDKVEYRTAILPGCP IDSNAYTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCP IDSNAYTYPVALSCHCGACRTDSDECAHRAS-TSHβStoklebackDKVEYRTAILPGCP IDSNAYTYPVALSCHCGACRTDSDECAHRAS-TSHβSblefishDKVEYRTAILPGCP IDSNAYTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCP IDSNAYTYPVALSCHCGACRTDSDECAHRAS-	INTTICSGSCLTRDANVKRLLPKSALSONICTF
TSHβ2Little skateMCCSMRVLSLLPLLLSLAARAQCSLTRHVLYVEKEGCNFCMATSHβ2LungfishMNCLWLLPAVLLHCRPVGSLCTMSRYMLYIEREECSHCMATSHβ2CoelacanthMNFTWLVPVVICMSCTSVNSLCTVTRYMMYVEKEQCSHCIALHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLLPCEPINETISVEKDGCPKCLVFSHβEuropean eelRDVVYETVHLPGCPSGMDLHFTYPVALSCECSKCNTDSTDCGPLNT-TSHβ3SablefishHSLVYRAHVPGCPQDVNSQLYYPAAQRCSCRRCDTRTHHCVRTRR-TSHβ3SablefishHSLVYRAHVPGCPQDVNSQLYYPAAQRCSCRRCDTRTHHCVRTRR-TSHβ3SlucklebackLSLVYRAVHLPGCPPQVNPQVYPAAHCLCKRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRVAHIPGCPPDVNAELYPVALCSCRCDTRTHHCVQRRR-TSHβ3Atlantic codGSLVYRTARLVGCPRVNPVYYPAHCCSCRCDTRTHHCVQKSR-TSHβ3European eelQSLVYHAVSLPGCPPDVDPLFSPVALRCHCSRCNTSNTECLHRGK-TSHβEuropean eelQAVEYRTARLPGCPPHVDPRFSYPVALHCTCRACDPARDECTHRAS-TSHβEuropean eelQAVEYRTALPGCPSHDPHFTYPVALSCHCGACNTAVDECAHRAS-TSHβAtlantic codNQVEYRTALLPGCPISDPVFSYPVALSCHCGACRTDRDECTHRAS-TSHβAtlantic codNQVEYRTALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβAtlantic codNQVEYRTALLPGCPINANPVFTYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTALLPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSticklebackDKVEYRTALLPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβShotfeishDKVEYRTALLPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSticklebackDKVEYRTALLPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβStoklebackDKVEYRTALLPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-<	INTTVCAGYCMSRDVNIKTLLPKNALVONVCTF
TSHβ2LungfishMNCLWLLPAVLLLHCRPVGSLCTMSRYMLYIEREECSHCMATSHβ2CoelacanthMNFTWLVPVVICMSCTSVNSLCTVTRYMMYVEKEQCSHCIALHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLLPCEPINETISVEKDGCPKCLVFSHβEuropean eelRDVVYETVHLPGCPSGMDLHFTYPVALSCECSKCNTDSTDCGPLNT-TSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYYPAAQRCSCRRCDTRTHHCVRTRR-TSHβ3Bluefin tunaLSLVYQAVHFPGCPQNNPQUYFPVALCSCRCDSRTHHCVRSSQ-TSHβ3SticklebackLSLVYRAAHLPGCPPDVNAELYPVALCSCRCDTRTHHCVRTSR-TSHβ3Atlantic codGSLVYRAARLVGCPRNNPVIYYPEFHRCKCRSCDRRTHHCVQKSR-TSHβ3European eelQSLVYRAXLVGCPRNNPVIYYPEFHRCKCRSCDRRTHHCVQKSR-TSHβ3European eelQAVEYRTAELPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-TSHβ4European eelQAVEYRTAELPGCPPHVDPRFSYPVALHCTCRACDPARDECTHRAS-TSHβEuropean eelQAVEYRTAELPGCPIDSDPVFSYPVALSCHCGACNTAVDECAHRAS-TSHβAtlantic codNQVEYRTAILPGCPIDSDPVFSYPVALSCHCGACNTAVDECAHRAS-TSHβAtlantic codNQVEYRTAILPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTAILPGCPIDDNPFTYPVALSCHCGACRTDSDECAHRAS-TSHβSticklebackDKVEYRTAILPGCPIDNNVFTYPVALSCHCGACRTDSDECAHRAS-SHβStokleganQULSYHTVILPGCPLHSNPSYSAVAMSCRCRCNTDYSECTMEPTSHβSturgeonQDLSYHTVILPGCPLHSNPSYSAVAMSCRCRCNTDYSECTMEPTSHβElephant sharkDKVEYRTVILPGCPDIDPFYRLPVLGCVCSQCSTDTDCTTCVTSHβ2LingfishNTIKYMTMILPGCPPDVDPYRLPVLGCVCSQCSTDTDCTTCVTSHβ2LingfishNTIKYMTMILPGCPPDVDPYRLFAVATSCKCSQCND	VNTTICAGFCMSQDVNIKALLPKMALAQRVCTY
TSHβ2 CoelacanthMNFTWLVPVVICMSCTSVNSLCTVTRYMMYVEKEQCSHCIALHβ European eel/YPECTWLLFVCLCHLLVSAGGSLLLPCEPINETISVEKDGCPKCLVFSHβ European eelRDVVYETVHLPGCPSGMDLHFTYPVALSCECSKCNTDSTDCGPLNT-TSHβ3 SablefishHSLVYRAAHVPGCPQDVNSQLYYPAAQRCSCRCDTRTHHCVRTRR-TSHβ3 Subefin tunaLSLVYQAVHFPGCPQDVNSQLYPAAQRCSCRCDTRTHHCVRTSR-TSHβ3 SticklebackLSLVYRAVHLPGCPPGVNPQVYPAAHRCLCKRCDTRTHHCVRTSR-TSHβ3 Atlantic codGSLVYRTARLVGCPRNVNPVIYPEFHRCKCRSCDRRTHHCVQSR-TSHβ3 Atlantic codGSLVYRTARLVGCPRNVNPVIYPEFHRCKCRSCDRTHHCVQSR-TSHβ3 European eelQSLVYHAVSLPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-TSHβ European eelQAVEYRTARLPGCPPHVDPRFSYPVALHCTCRACDPARDECTHRAS-TSHβ Atlantic codNQVEYRTALLPGCPSEGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβ Atlantic codNQVEYRTALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECALRRA-TSHβ Atlantic codNQVEYRTALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECALRRAS-TSHβ MedakaDKVEYRTALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECALRRAS-TSHβ SticklebackDKVEYRTALLPGCPIDNAVFTYPVALSCHCGACRTDSDECALRRAS-TSHβ SbicklebackDKVEYRTALLPGCPINANPVFTYPALSCHCGACRTDSDECAHRAS-TSHβ Sbotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβ SturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNDTYSCTMEPTSHβ Elephant sharkDELEYRTVRIPGCPPDVDPYRLPVULSCQCSQCATETTDCTNDIANTSHβ2 LingfishNTIKYMTRLPGCPPDVDPYRLPVULSCQCSQCATETTDCTNDIANTSHβ2 LungfishNTIKYMTRLPGCPPDVDPYRLPVULSCCSQCATETTDCTNDENTSHβ2 LingfishNTIKYMTRLPGCPPDVDPYRLFAVATSCKCSQCATETTDCTNDECTNEG	INTTICTGYCMTRDPNVKILIPKSVLSQNVCTY
LHβEuropean eel/YPECTWLLFVCLCHLLVSAGGSLLLPCEPIN ETISVEKDGCPKCLVFSHβEuropean eelRDVVYETVHLPGCPSGMDLHFTYPVALSCECSKCNTDSTDCGPLNT-FSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYYPAAQRCSCRRCDTRTHHCVRTRR-TSHβ3Bluefin tunaLSLVYQAVHFPGCPQNNPQLYFPVALTSHβ3SticklebackLSLVYRAHLPGCPPGVNPQVYPAAHRCLCKRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRVAHIPGCPPDVNAELYPVALTSHβ3Atlantic codGSLVYRTARLVGCPRNVNPVIYPEFHRCKCRSCDRTHHCVQKSR-TSHβ3European eelQSLVYHAVSLPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-TSHβ3European eelQAVEYRTAELPGCPPHVDPFSYPVALRCHCSRCNTSNTECLHRGK-TSHβEuropean eelQAVEYRTAELPGCPENDPFFSYPVALSCHCGACNTAVDECAHRAS-TSHβAtlantic codNQVEYRTAILPGCPSEGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECHRAS-TSHβSticklebackDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSblefishDKVEYRTAILPGCPINANPYTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPYTYPVALSCHCGACRTDSDECAHRAS-TSHβSblefishDKVEYRTAILPGCPINANPYTYPVALSCHCGACRTDSDECAHRAS-TSHβSblefishDKVEYRTAILPGCPINANPYTYPVALSCHCGACRTDSDECAHRAS-TSHβSturgeonQDLSYHTVLPGCPHNSPHSYSYAVAMSCRCRKCNTDYSECTMEPTSHβSturgeonQDLSYHTVLPGCPHNSPHSYSYAVAMSCRCRKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPPDIDFYRLPVVLSCCCSQCATETTDCTNDIANTSHβ2LingishNTIKYMTMLPGCPPDUDPYRFAVATSCKCSQCNTDTTDCTNGVTSHβ2LingishNTIKYMTRL	INTTICSGYCITRDPNLKALLPRTALSOSVCTY
FSHβ European eel       RDVVYETVHLPGCPSGMDLHFTYPVALSCECSKCNTDSTDCGPLNT-         TSHβ3 Sablefish       HSLVYRAAHVPGCPQDVNSQLYYPAAQRCSCRCDTRTHHCVRTRR-         TSHβ3 Bluefin tuna       LSLVYQAVHFPGCPQNVNPQLYFPAALCSCRCDTRTHHCVRTSR-         TSHβ3 Stickleback       LSLVYRAVHLPGCPPGVNPQVYPAAHRCLCKRCDTRTHHCVRTSR-         TSHβ3 Atlantic cod       GSLVYRTARLVGCPRNVNPVIYYPAHRCSCRCDTRTHHCVQKSR-         TSHβ3 Atlantic cod       GSLVYRTARLVGCPRNVNPVIYYPEHRCKCRSCDRRTHHCVQKSR-         TSHβ3 European eel       QSLVYHAVSLPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-         TSHβ European eel       QAVEYRTAELPGCPPHVDPRFSYPVALHCTCRACDPARDECTHRAS-         TSHβ Zebrafish       QEVEYRTAVLPGCPSGSSLFSYPVALSCHCSTCKTHSDECALRTR-         TSHβ Medaka       DKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACNTAVDECAHRAS-         TSHβ Medaka       DKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPG         TSHβ Stickleback       DKVEYRTAILPGCPIDSDPVFSYPVALSCHCGACRTDSDECAHRAS-         TSHβ Bluefin tuna       DKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-         TSHβ Sablefish       DKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-         TSHβ Sturgeon       QULSYHTVTLPGCPLHINPLLSYPLALRCHCSRCNTDVNDCTHKA         TSHβ Sturgeon       QULSYHTVTLPGCPHVSPHYSYPVARSCSCCKCNTDYTDCTHET         TSHβ Elephant shark       DELEYRTVRIPGCPPUDPFYRLPVVLSCQCSQCATETTDCTNDIAN         TSHβ Elephant shark       HNIRYMMRLPGCPPDUDPFYRLPVVLSCQCSQCSTDTTDCTNQEN-     <	FOTSICSGHCITKDPSYKSPLSTVYORVCTY
FSHβEuropean eelRDVVYETVHLPGCPSGMDLHFTYPVALSCECSKCNTDSTDCGPLNT-TSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYYPAAQRCSCRRCDTRTHHCVRTRR-TSHβ3Bluefin tunaLSLVYQAVHFPGCPQNVNPQLYFPVALCSCRRCDTRTHHCVRTSR-TSHβ3Bluefin tunaLSLVYQAVHFPGCPQNVNPQLYFPVALCSCRRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRVAHLPGCPPGVNPQVYYPAAHRCLCKRCDTRTHHCVQPRR-TSHβ3MedakaLSLVYRVAHIPGCPPDVNAELYYPVAHCSSCRCDTRTYHCVQPRR-TSHβ3Atlantic codGSLVYRTARLVGCPRNVNPVI YYPEFHRCKCRSCDRTHHCVQKSR-TSHβ3European eelQSLVYHAVSLPGCPPDVDPLFSPPVALRCHCSRCNTSNTECLHRGK-TSHβEuropean eelQAVEYRTAELPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-TSHβEuropean eelQAVEYRTAELPGCPPHVDPRFSYPVALHCTCRACDPARDECTHRAS-TSHβEuropean eelQAVEYRTALPGCPSEGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRTAILPGCPSEGSSLFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTAILPGCPIDSDPVFSYPVALSCHCGACRTDSDECHRAS-TSHβSablefishDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSbotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPHVSPHYSYPVARSCSCDKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPPDIDPFYRLPVVLSQCSQCATETTDCTNDIANTSHβ2Elephant sharkHNIRYMMIRLPGCPPDIDPFYRLPVVLSQCSQCATETTDCTNDCINGATSHβ2Little skateRHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNDTTDCTNGATSHβ2LonglishNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNDTTDCTNGA-	
FSHβEuropean eelRDVVYETVHLPGCP SGMDLHFTYPVALSCECSKCNTDSTDCGPLNT-TSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYYPAAQRCSCRRCDTRTHHCVRTRR-TSHβ3Bluefin tunaLSLVYQAVHFPGCPQNVNPQLYFPVALCSCRRCDTRTHHCVRTSR-TSHβ3SticklebackLSLVYRAHLPGCPPGVNPQVYPAAHRCLCKRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRVAHLPGCPPDVNAELYYPVAHCSSCRCDTRTHHCVQFRR-TSHβ3Atlantic codGSLVYRTARLVGCPRNVNPV1YYPEFHRCKCRSCDRTHHCVQKSR-TSHβ3European eelQSLVYRARLPGCPPUNDPLFYPVALRCKCRSCDRTHHCVQKSR-TSHβ4European eelQAVEYRTARLPGCPPUNDPLFSPVALRCHCSRCNTSNTECLHRGK-QSLVYHAVSLPGCPPUNDPRFSYPVALHCTCRACDPARDECTHRAS-QSLVYHAVSLPGCPPUNDPRFSYPVALHCTCRACDPARDECTHRAS-TSHβEuropean eelQAVEYRTALPGCPSEGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβAtlantic codNQVEYRTAILPGCPSEGSSLFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTAILPGCPIDDNAVFTYPVALSCHCGACRTDSDECHRAS-TSHβSablefishDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSbotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβElephant sharkDELEYRTVRIPGCPPDIDPFYRLPVVLSCQCSQCATETTDCTNDIANTSHβ2Elephant sharkHNIRYMMIRLPGCPPDIDPFYRLPVVLSCQCSQCATETTDCTTQCTSHβ2Little skateRHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGATSHβ2Little skateNTIKYMTMRLPGCPPD	>
TSHβ3SablefishHSLVYRAAHVPGCPQDVNSQLYYPAAQRCSCRRCDTRTHHCVRTRR-TSHβ3Bluefin tunaLSLVYQAVHFPGCPQNVNPQLYFPVALCSCRRCDTRTHHCVRTSR-TSHβ3SticklebackLSLVYRAVHLPGCPPGVNPQVYYPAAHRCLCKRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRVAHIPGCPPDVNAELYYPVAHCSCRCDTRTHHCVQFRR-TSHβ3Atlantic codGSLVYRTARLVGCPRNVNPVIYYPEFHRCKCRSCDRTHHCVQKSR-TSHβ3ZebrafishRSLVYRSARMPGCPVHIDPLFYPVARCNCTKCNTSRNECVFRHK-TSHβ3European eelQAVEYRTARLPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-QSLVYHAVSLPGCPPDVDPFSYPVALHCTCRACDPARDECTHRAS-QEVEYRTAVLPGCPSEGSSLFSYPVALSCHCSACNTAVDECAHRAS-TSHβZebrafishQEVEYRTALLPGCPSEGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRTAILPGCPIDDNAVFTYPVALSCHCGACRTDSDECVHRAPGTSHβSablefishDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSbotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβElephant sharkDELEYRTVRIPGCPPDIDFYRLPVVLSCQCSQCATETTDCTNQEN-TSHβ2Elephant sharkHNIRYMMIRLPGCPPDIDPFYRLPVVLSCQCSQCATETTDCTNQEN-TSHβ2Little skateRHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGATSHβ2Little skateNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGATSHβ2LonglishNTIKYMTMRLPGCPPDVDPYYHFAVATSCK	-EVSG <mark>C</mark> LTH*
TSHβ3Bluefin tunaLSLVYQAVHFPGCPQNVNPQLYFPVALCSCRRCDSRTHHCVRSSQ-TSHβ3SticklebackLSLVYRAVHLPGCPPGVNPQVYYPAAHRCLCKRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRVAHIPGCPPDVNALLYYPVAHCSCRCDTRTHHCVQPRR-TSHβ3Atlantic codGSLVYRTARLVGCPRNVNPVIYYPEFHRCKCRSCDRTHHCVQKSR-TSHβ3ZebrafishRSLVYRSARMPGCPVHIDPLFFYPVARCNCTKCNTSRNECVFRHK-TSHβ3European eelQAVEYRTARLPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-TSHβEuropean eelQAVEYRTARLPGCPPHVDPRFSYPVALHCTCRACDPARDECTHRAS-TSHβZebrafishQEVEYRTAVLPGCPSHADPHFTYPVALSCHCSTCKTHSDECALRTR-TSHβAtlantic codNQVEYRTAILPGCPEDSDPVFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSbotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPHVSPHSYSYAVAMSCRCRKCNTDYSECTMEPTSHβSturgeonQDLSYHTVTLPGCPHVSPHSYPVARSCSCDKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPPDIDPFYRLPVULSCQCSQCATETTDCTNDIANTSHβ2Little skateRHMDYISIRLPGCPPDIDPFYRLPVVLSCQCSQCATETTDCTNDIANTSHβ2Little skateRHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGATSHβ2CoelacanthNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGATSHβ2Little skateRHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGA	ISHDG <mark>C</mark> TVTL-GGVRSQTQPAVGT*
TSHβ3SticklebackLSLVYRAVHLPGCPPGVNPQVYYPAAHRCLCKRCDTRTHHCVRTSR-TSHβ3MedakaLSLVYRVAHIPGCPPDVNAELYYPAHCCSCRCDTRTHHCVQPRR-TSHβ3Atlantic codGSLVYRTARLVGCPRNVNPVIYPEFHRCKCRSCDRRTHHCVQKSR-TSHβ3ZebrafishRSLVYRSARMPGCPVHIDPLFYPVARRCNCTKCNTSRNECVFRHK-TSHβ3European eelQAVEYRTARLPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-TSHβEuropean eelQAVEYRTARLPGCPPHVDPRFSYPVALRCCTCRACDPARDECTHRAS-TSHβZebrafishQEVEYRTAVLPGCPSHADPHFTYPVALSCHCSTCKTHSDECALRTR-TSHβAtlantic codNQVEYRTAILPGCPSSQSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSpotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPPTGVSSQHSYTALSCKCKNCDTDYTDCTNQEN-TSHβ2Little skateRHMDYISIRLPGCPPDIDFYRLPVVLSCQCSQCATETTDCTNDIANTSHβ2Little skateRHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGATSHβ2CoelacanthNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGANKVKYLTIRIPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGANKKYLTIRIPGCAPHDPYYHFAVATSCKCSQCNTDTTDCTNGA	IPHDRCTMAL-GSLKNENQPALET*
TSHβ3MedakaLSLVYRVAHIPGCPPDVNAELYYPVAHCSCRCCDTRTYHCVQPRR-TSHβ3Atlantic codGSLVYRTARLVGCPRNVNPVIYYPEFHRCKCRSCDRRTHHCVQKSR-TSHβ3ZebrafishRSLVYRSARMPGCPVHIDPLFYPVARCNCTKCNTSRNECVFRHK-TSHβ3European eelQAVEYRTAELPGCPPDVDPLFSFPVALRCHCSRCNTSNTECLHRGK-TSHβZebrafishQEVEYRTAELPGCPPHVDPRFSYPVALRCCTCRACDPARDECTHRAS-TSHβAtlantic codNQVEYRTAILPGCPSHADPHFTYPVALSCHCSTCKTHSDECALRTR-TSHβAtlantic codNQVEYRTAILPGCPSSSSIFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβMedakaDKVEYRTAILPGCPIDSDPVFSYPVALSCHCGACRTDSDECHRAPGTSHβSticklebackDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSpotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβSturgeonQDLSYHTVTLPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPPDIDFYRLPVULSCQCSQCATETTDCTNDIANTSHβ2Little skateRHMDYISIRLPGCPPDIDPFYRLPVULSCQCSQCATETTDCTNDIANTSHβ2Little skateRHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGATSHβ2CoelacanthNTIKYMTMRLPGCPPDVDPYYHFAVAINCKCSLCNTDSTDCTNGANKVKYLTIRIPGCPPDVDPYYHFAVAINCKCSLCNTDSTDCTNEGNKKYLTIRIPGCPPDVDPYYHFAVAINCKCSLCNTDSTDCTNGA	/STEGCSATL-DGVKSQTQPSVATQQYVNTGD/
TSHβ3Atlantic codGSLVYRTARLVGCPRNVNPVIYYPEFHRCKCRSCDRRTHHCVQKSR- TSHβ3TSHβ3ZebrafishRSLVYRSARMPGCPVHIDPLFYPVARCNCTKCNTSKNECVFRHK- QSLVYHAVSLPGCPPDVDPLFSYPVALRCHCSRCNTSNTECLHRGK- TSHβTSHβEuropean eelQAVEYRTAELPGCPPHVDPRFSYPVALHCTCRACDPARDECTHRAS- QEVEYRTALPGCPSHADPHFTYPVALSCHCSTCKTHSDECALRTR- QEVEYRTALLPGCPSGSSLFSYPVALSCHCGACNTAVDECAHRAS- DKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACNTAVDECAHRAS- TSHβTSHβMedakaDKVEYRTAILPGCPSGSSLFSYPVALSCHCGACNTAVDECAHRAS- DKVEYRTAILPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPG DKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECHRAS- TSHβTSHβSticklebackDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECHRAS- DKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS- TSHβTSHβSablefishDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS- DKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS- TSHβTSHβSablefishDKVEYRTAILPGCPIDNAPVFTYPVALSCHCGACRTDSDECAHRAS- DKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS- TSHβTSHβSablefishDKVEYRTAILPGCPIDNAVFTYPVALSCHCGACRTDSDECAHRAS- DKVEYRTAILPGCPPHVSPHYSYVAAMSCRCRKCNTDYSECTMEP TSHβTSHβSturgeonQDLSYHTVTLPGCPHVSPHYSYVARSCSCDKCNTDYTDCTHET DSHβTSHβElephant sharkDELEYRTVRIPGCPPGVSQHSYPTALSCKCKNCDTDYTDCTVQEN- HSHβ2TSHβ2Little skateRHMDYISIRLPGCPPDIDPFYRLPVVLGCVCSQCSTDTTDCTTGV NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGA NKVKYLTIRIPGCAPHUDPYYHFAVATSCKCSQCNTDTTDCTNGA NKVKYLTIRIPGCAPHUDPYYHFAVAINCKCSLCNTDSTDCTNEG	FSYDQ <mark>C</mark> SVKL-GSGGHQNEN <mark>C</mark> FG <mark>N</mark> ITN <mark>C</mark> *
TSHβ3ZebratishRSLVYRSARMPGCPVHIDPLFFYPVARRCNCTKCNTSRNECVFRHK- QSLVYHAVSLPGCPPDVDPLFSPVALRCHCSRCNTSNTECUHRGK-TSHβEuropean eelQAVEYRTAELPGCPPHVDPRFSYPVALRCHCSRCNTSNTECLHRGK- QEVEYRTAELPGCPPHVDPRFSYPVALSCHCSCKTHSDECALRTR- SHβ Atlantic codTSHβQEVEYRTAVLPGCPSHADPHFTYPVALSCHCGACNTAVDECAHRAS- QEVEYRTALLPGCPSGSSLFSYPVALSCHCGACNTAVDECAHRAS- DKVEYRTALLPGCPIDSDPVFSYPVALSCRCGTCRTDSDECVHRAPGTSHβMedakaDKVEYRTALLPGCPIDSDPVFSYPVALSCRCGACRTDRDECTLRLN- DKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS- TSHβTSHβSablefishDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS- DKVEYRTAILPGCPIDNAPVFTYPVALSCHCGACRTDSDECAHRAS- TSHβTSHβSablefishDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS- DKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS- TSHβTSHβSablefishDKVEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKA DLSYHTVTLPGCPHVSPHYSYPVARSCSCDKCNTDYTDCTHET TSHβTSHβSturgeonQDLSYHTVTLPGCPHVSPHYSYPVARSCSCDKCNTDYTDCTHET TSHβTSHβElephant sharkDELEYRTVRIPGCPPTGVSSQHSYPTALSCKCKNCDTDYTDCTVQEN- HNIRYMMIRLPGCPPDIDPFYRLPVVLSQCSQCATETTDCTNDIAN TSHβ2TSHβ2Little skateRHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGA NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGA NKVKYLTIRIPGCAPHIDPYYNFAVAINCKCSLCNTDSTDCTNEG	YPLNQCRKTR-HKRKGKDSN*
ISHβ3European eelQSLVYHAVSLPGCPPDVDPLFSPVALRCHCSRCMTSNTECLHRGK-TSHβEuropean eelQAVEYRTAELPGCPPHVDPRFSYPVALRCHCSRCMTSNTECLHRGK-TSHβZebrafishQEVEYRTALPGCPSHADPHFTYPVALSCHCSTCKTHSDECALRTR-TSHβAtlantic codNQVEYRTALLPGCPSGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTAILPGCPIDSDPVFSYPVALSCHCGACRTDSDECTLRLN-TSHβBluefin tunaDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSpotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβElephant sharkDELEYRTVRIPGCPFUSSQHSYPTALSCKCKNCDTDYTDCTHETTSHβ2Elephant sharkHNIRYMMIRLPGCPPDIDPFYRLPVVLSCQCSQCATETTDCTNDIANTSHβ2Little skateRHMDYISIRLPGCPPDIDPFYRLPVVLSCQCSQCATETTDCTTQETSHβ2Little skateNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGATSHβ2CoelacanthNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGA	HKHNRCSKQL-RTV*
TSHβEuropean eelQAVEYRTAELPGCPPHVDPRFSYPVALHCTCRACDPARDECTHRAS- QEVEYRTAVLPGCPSHADPHFTYPVALSCHCSTCKTHSDECALRTR-TSHβAtlantic codNQVEYRTALLPGCPSGSSLFSYPVALSCHCGACNTAVDECAHRAS- DKVEYRTALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECTLRLN- DKVEYRTALLPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS- TSHβTSHβSablefishDKVEYRTALLPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS- QVLEYRTALLPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS- TSHβTSHβSablefishDKVEYRTALLPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS- QVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKA TSHβTSHβSopotted garQVLEYRTVLPGCPLHSNPSYSVAVAMSCRCRKCNTDYSECTMEP KDIEYNSVTVPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTHET TSHβTSHβElephant sharkDELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDDTYTDCTVQEN- HIRYMMIRLPGCPPDIDPFYRLPVVLSQCSQCATETTDCTNDIAN RHMDYISIRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTGV NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGA NKVKYLTIRIPGCPPHDPYRFAVAINCKCSLCNTDSTDCTNEG	RLPNPCDSTL-CYAKGPPKAATATSLTGTYQE/
TSHβZebraishQEVEYRTAVLPGCPSHADPRFTYPVALSCHCSTCKTHSDECALRTR-TSHβAtlantic codNQVEYRTAILPGCPSEGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRAALLPGCPIDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTAILPGCPIDSDPVFYPVALSCHCGACRTDSDECAHRAS-TSHβBluefin tunaDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPIDTNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSpotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβCoelacanthKDIEYNSVTVPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPFUSSQBSYPTALSCKCKNCDDYTDCTVQEN-TSHβ2Little skateRHMDYISIRLPGCPPDIDFYRLPVVLSQCSQCATETTDCTNDIANTSHβ2LongfishNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTNGANKVKYLTIRIPGCPPIDVYRFAVAINCKCSLCNTDSTDCTNGANKVKYLTIRIPGCPPIDPYRFAVAINCKCSLCNTDSTDCTNGA	ADGDRCSKPLLLHMHAYPGQSNYIQTL*
TSHβAttantic codNQVEYRTALLPGCPJSEGSSLFSYPVALSCHCGACNTAVDECAHRAS-TSHβMedakaDKVEYRAALLPGCPJDSDPVFSYPVALSCHCGACRTDSDECVHRAPGTSHβSticklebackDKVEYRTALLPGCPJDSDPVFSYPVALSCHCGACRTDSDECAHRAS-TSHβBluefin tunaDKVEYRTAILPGCPJDSNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPJDNAVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPJINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPJINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPJINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSopted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβCoelacanthKDIEYNSVTVPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTVQEN-TSHβElephant sharkDELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDTDYTDCTVQEN-TSHβ2Little skateRHMDYISIRLPGCPPDIDFYRLPVVLGCVCSQCSTDTTDCTTQTSHβ2LongfishNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCINGANKVKYLTIRIPGCPPHUDPYYBFAVAINCKCSLCNTDSTDCTNEGNKVKYLTIRIPGCPPHDPYYBFAVAINCKCSLCNTDSTDCTNEG	SAGMRCSKPV-HHLYPEENNYAQAYWDQYE*
TSHβDKVEYRAALLPGCP1D5DPVFSYPVALSCRCGTCRTD5DECVHRAPGTSHβDKVEYRTVILPGCA1DSNPAYTYPVALSCRCGACRTDSDECTLRLN-TSHβDKVEYRTAILPGCP1DTNAVFTYPVALSCRCGACRTDSDECAHRAS-TSHβDKVEYRTAILPGCP1NANPVFTYPVALSCRCGACRTDSDECAHRAS-TSHβDKVEYRTAILPGCP1NANPVFTYPVALSCRCGACRTDSDECAHRAS-TSHβDKVEYRTAILPGCP1NANPVFTYPVALSCRCGACRTDSDECAHRAS-TSHβDKVEYRTVLPGCPLHINPLLSYPLALRCHCSRCNTDNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβCoelacanthKDIEYNSVTVPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDTDYTDCTVQEN-TSHβ2Elephant sharkHNIRYMMIRLPGCPPDIDFFYRLPVLSCQCSQCATETTDCTNDIANTSHβ2LungfishNTIKYMTMRLPGCPPDVDPYYRLPVLGCVCSQCSTDTTDCTNGATSHβ2CoelacanthNKVKYLTIRIPGCPHDVDFYRFAVAINCKCSLCNTDSTDCTNEG	SNRPTCTKPV-RHIYQSNFLLPF*
TSHβSticklebackDKVEYRTVILPGCAIDSNPATTYPVAISCHCGACRTDRDECTLRLN-TSHβBluefin tunaDKVEYRTAILPGCPIDTNAVFTYPVAISCHCGACRTDSDECAHRAS-TSHβSablefishDKVEYRTAILPGCPINANPVFTYPVALSCHCGACRTDSDECAHRAS-TSHβSpotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβCoelacanthKDIEYNSVTVPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDTJYTDCTVQEN-HNIRYMNIRLPGCPPDIDPFYRLPVVLSCQCSQCATETTDCTNDIANNHMYISIRLPGCPPODPYYRLPVVLGCVCSQCSTDTTDCTTGVTSHβ2LungfishNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCINGANKVKYLTIRIPGCPPUDPYYRFAVAINCKCSLCNTDSTDCTNEGNKVKYLTIRIPGCPHDPYYRFAVAINCKCSLCNTDSTDCTNEG	VGGARCTKPV-RRIYPYPGQSTYMTPF*
TSHβDidemit titlaDKVEIRTAILPGCPIDINARY FIPVALSCHCGACRTDSDECAHRAS-TSHβSablefishDKVEIRTAILPGCPINANPVFTIPVALSCHCGACRTDSDECAHRAS-TSHβSpotted garQVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDNDCTHKATSHβSturgeonQDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEPTSHβCoelacanthKDIEYNSVTVPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTHETTSHβElephant sharkDELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDTDYTDCTVQEN-TSHβ2Elephant sharkHNIRYMMIRLPGCPPDIDFYRLPVLSCQCSQCATETTDCTNDIANTSHβ2Little skateRHMDYISIRLPGCPPOVDPYYRLPVVLGCVCSQCSTDTTDCTTGVTSHβ2CoelacanthNTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCINGATSHβ2CoelacanthNKVKYLTIRIPGCPPDVDPYYFFAVAINCKCSLCNTDSTDCTNEG	SYDANCARPV-RRVYPYPGQSNYMIPF*
TSHβ       Spotted gar       QVLEYRTVLLPGCPLHINPLLSYPLALRCHCSRCNTDDNDCTHKA         TSHβ       Sturgeon       QDLSYHTVTLPGCPLHINPLLSYPLALRCHCSRCNTDNNDCTHKA         TSHβ       Sturgeon       QDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEP         TSHβ       Coelacanth       KDIEYNSVTVPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTHET         TSHβ       Elephant shark       DELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDTDYTDCTVQEN-         TSHβ2       Elephant shark       HNIRYMMIRLPGCPPDIDFYRLPVVLSCQCSQCATETTDCTNDIAN         TSHβ2       Little skate       RHMDYISIRLPGCPPOVDPYYRLPVVLGCVCSQCSTDTTDCTTGV         TSHβ2       Coelacanth       NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCINGA         NKVKYLTIRIPGCPHDVDYYRFAVAINCKCSLCNTDSTDCTNEG       NKVKYLTIRIPGCPPHDPYYRFAVAINCKCSLCNTDSTDCTNEG	VDGARCIAPV-RALIPIPGQSNIMIPF*
TSHβ       Sturgeon       QDLSYHTVTLPGCPLHSNPSYSYAVAMSCRCRKCNTDYSECTMEP         TSHβ       Coelacanth       KDIEYNSVTVPGCPPHVSPHYSYPVARSCSCDKCNTDYTDCTHET         TSHβ       Elephant shark       DELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDTDYTDCTVQEN-         TSHβ2       Elephant shark       HNIRYMMIRLPGCPPDIDFYRLPVLSCQCSQCATETTDCTNDIAN         TSHβ2       Little skate       RHMDYISIRLPGCPPGVDPVYRLPVLGCVCSQCSTDTTDCTTGV         TSHβ2       Longfish       NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCINGA         TSHβ2       Coelacanth       NKVKYLTIRIPGCPHDVPYKFAVAINCKCSLCNTDSTDCTNEG	SETNECTRE -ODADSVDCOSNVIOLD*
TSHβ Coelacanth       KDIEYNSVTVPGCPPHVSPHVSPVARSCSCDKCNTDYDCTHET         TSHβ Clephant shark       DELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDTDYDCTVQEN-         TSHβ2 Elephant shark       HNIRYMMIRLPGCPPDIDPFYRLPVUSCQCSQCATETTDCTNDIAN         TSHβ2 Little skate       RHMDYISIRLPGCPPOVDPYYRLPVUSCQCSQCSTDTTDCTTGV         TSHβ2 Longfish       NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCINGA         TSHβ2 Coelacanth       NKVKYLTIRIPGCPPUDPYYRFAVAINCKCSLCNTDSTDCTNEG	LEASPCTKPI-QFADSIFGQSNFIQLD"
TSHβ         Elephant shark         DELEYRTVRIPGCPTGVSSQHSYPTALSCKCKNCDTDYTDCTVQEN-           TSHβ2         Elephant shark         HNIRYMMIRLPGCPPDIDPFYRLPVVLSCQCSQCATETTDCTNDIAN           TSHβ2         Little skate         RHMDYISIRLPGCPPGVDPVYRLPVVLGCVCSQCSTDTTDCTTGV           TSHβ2         Lungfish         NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTIGA           TSHβ2         Coelacanth         NKVKYLTIRIPGCAPHIDPYYRFAVAINCKCSLCNTDSTDCTNEG	VRTNHCIKPT-VASPLKLF*
TSHβ2         Elephant shark         HNIRYMIRLPGCPPDIDPFYRLPVUSCQCSQCATETTDCTNDIAN           TSHβ2         Little skate         RHMDYISIRLPGCPPGVDPVYRLPVUSCQCSQCSTDTTDCTTGV           TSHβ2         Lungfish         NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCTIGA           TSHβ2         Coelacanth         NKVKYLTIRIPGCAPHIDPYYRFAVAINCKCSLCNTDSTCTNEG	LEANVCREPO-SETNSOD*
TSHβ2         Little skate         RHMDYISIRLPGCPPGVDPVYRLPVVLGCVCSQCSTDTTDCTTGV           TSHβ2         Lungfish         NTIKYMTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCINGA           TSHβ2         Coelacanth         NKVKYLTIRIPGCAPHIDPYYRFAVAINCKCSLCNTDSTDCTNEG	ONPYHCTKPO-WRIPATNSRIFIL*
TSHβ2 Lungfish NTIK¥MTMRLPGCPPDVDPYYHFAVATSCKCSQCNTDTTDCINGA TSHβ2 Coelacanth NKVK¥LTIRIPGCAPHIDPYYRFAVAINCKCSLCNTDSTDCTNEG	EAHLHCAKPO-WHIPASRSPLLLFSATRRKOE/
TSHB2 Coelacanth NKVKYLTIRIPGCAPHIDPYYRFAVAINCKCSLCNTDSTDCTNEG	EATIOCSKPO-WRIPASNSRILLIO*
	ENPNECNOPO-WRIPAMKSRLLLI*
LHβ European eel RDVR¥ETVRLPDCRPGVDPHVTFPVALSCDCNLCTMDTSDCAIQS	LRPDFCMSOR-ASLPA*
	THENT NOLO-MKILWWK2KPPPI,

**Figure 2. TSH**//-related sequence alignment. Amino acid sequence alignment of representatives of the three groups of TSH $\beta$  subunits. Eel LH $\beta$  and FSH $\beta$  sequences are given for comparison. The predicted signal peptide is highlighted in blue. The exon splicing site is indicated by the red vertical bar. Overall conserved amino acids are in bold red. Specific positions in TSH $\beta$ 2 or - $\beta$ 3 sequences relative to TSH $\beta$  are highlighted in yellow. Potential glycosylation sites are highlighted in green. Additional, non-conserved cysteine residues are highlighted in red. Sequences might be truncated in the signal peptide or the carboxy-terminal end for convenience. Full-length sequences are presented in Fig. S1 and references are given in Table S1.

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vertebrate glycoprotein hormone  $\beta$  subunits is usually well conserved with two sites in FSH $\beta$  and most likely in the ancestral  $\beta$  subunit, whereas LH $\beta$  have kept one site (the one towards the Nterminal), and TSH $\beta$  the other. The  $Tsh\beta$  subunit precursor gene that was duplicated during the 3R encoded a subunit with only one glycosylation site found, at the second position, as for all classical TSH $\beta$  subunits. The additional site was then *de novo* created by mutation of a well-conserved aspartic acid (D) to an asparagine (N) at the first position, two amino acids upstream of a conserved threonine. Since glycosylation sites are more likely to be created by generating a serine or threonine residue downstream of an existing asparagine [43], there may be some kind of structural constraints for this glycosylation site to be re-created precisely at this position. Similar constraints should have applied for human LH $\beta$  subunit, also characterized by a glycosylation site that is, conversely, switched from the first position to the second, TSHtype position. Other scattered potential glycosylation sites are observed in some TSH $\beta$ 3 sequences (Fig. 2). Another feature of



**Figure 3. Phylogenetic tree of TSH** $\beta$  **related nucleotide sequences.** Phylogram of maximum likelihood relationships between *Tsh* $\beta$  coding sequences of representative species. The bootstrap values over 500 replicates (in %) are given next to each node in red (only the values above 50% are given). Cumulated distance values (from the node marked with a blue asterisk) are given in blue next to the species name for comparison of the estimated relative rate of evolution of teleost TSH $\beta$  and TSH $\beta$ 3 sequences (see Fig. S4 for the regression curve). *Tsh* $\beta$  gene references are given in Table S1.

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0.7

**Figure 4. Phylogenetic tree of TSHR-related amino acid sequences.** Phylogram of maximum likelihood relationships between TSHR amino acid sequences of representative species. The bootstrap values (in %) are given next to each node in red and the SH-like aLRT scores are given in black (when different from the boostrap value). Cumulated distance values (from the node marked with a blue asterisk) are given in blue next to the species name for comparison of the estimated relative rate of evolution of teleost TSHRa and TSHRb sequences (see Fig. S4 for the regression curve). *Tshr* gene references are given in Table S2. Black asterisks indicate partial sequences. doi:10.1371/journal.pone.0111361.g004

these TSH $\beta$ 3 sequences is the presence of additional cysteine residues, up to maximum of four in the medaka sequence. Whether these cysteine residues are involved in generating intraor inter-subunit disulfide bonds remains to be determined.

A single receptor for TSH and TSH2 in cartilaginous fish and basal lobe-finned fish

Only one receptor gene was identified in the coelacanth and elephant shark genomes, although they have two TSH $\beta$  subunits. It is thus likely that the additional TSH2 made up of TSH $\beta$ 2 and the common  $\alpha$  subunit, would act through binding to the same TSH receptor, as the classical TSH. Such a redundancy might have led to the loss of the second TSH in tetrapods and in rayfinned fish. The lungfish  $Tsh\beta 2$  subunit cDNA [37] as well as the two  $Tsh\beta$  cDNAs from the elephant shark [14] were cloned from pituitary libraries. In situ hybridization studies will be needed to determine whether they are produced by the same cells. Examination of tissue distribution could also reveal whether they are expressed in non-pituitary tissues.

### One or two potential receptors for TSH and TSH3 in teleosts

Two TSH receptor sequences were identified in most teleost groups (Fig. S3). Phylogenetic analysis (Fig. 4) showed that teleost TSHR are divided into two monophyletic groups, each one with one eel TSHR-type branching at a basal position. In contrast, only one TSHR was found in the spotted gar, confirming that the duplication of *Tshr* occurred early in the teleost radiation [20,21]. Synteny analysis revealed that one of the teleost duplicated receptors is conserved in the same chromosomal region as before the duplication event (Fig. 5). This is the *Tshra*-type receptor as previously named [21]. The eel *Tshrb* is maintained in a similar genomic region as *Tshra*, in agreement with it resulting from the 3R (strict double conserved synteny). In contrast, in more derived



**Figure 5. Syntenic analysis of TSHR-related genomic region.** Genomic region flanking *Tshr*-related genes were analysed in representative species (chromosome number is attached to the species name) by using the region overview on the Ensembl genome browser or by blast analysis on the eel assembled genome (see Fig. S2B for details). The phylogenetic relationships between the represented species are summarized on the right panel. *Tshrb* was translocated sometime between the emergence of eel and stickleback lineages. Genes are named according to the Ensembl nomenclature (Table S4). Gene positions are given (in Mega base) below the symbolized genes. doi:10.1371/journal.pone.0111361.g005



TSHRa and TSHRb compared structure

**Figure 6. Schematic representation of TSHRa and TSHRb sequence features.** Representation of the two types of teleost TSHR. LHR and FSHR are given for comparison. The exons splicing sites are indicated by a red dotted vertical bar. Both TSHR have conserved the typical architecture of the glycoprotein hormone receptor composed of a long extracellular domain comprising, from N- to C-terminal, a signal peptide (SP); a leucine-rich domain formed by successive leucine-rich repeat (LRR); a hinge region (in pink); a seven-transmembrane domain composed of seven helical regions (TM) connected by intracellular and extracellular loops alternatively and a cytosolic tail. Cysteine boxes at the N-terminal of the leucine rich domain and flanking the hinge are indicated (cb). Cysteine residues are in red, N-glycosylation sites in green. TSH-specific motif is in brown and sulfation site (YDY) in purple. Specific amino acids are labelled in blue. doi:10.1371/journal.pone.0111361.g006

teleost species such as cod, medaka and stickleback, Tshrb was found in a new genomic region (Fig. 5 and Table S4). This indicates that Tshrb was translocated some time after the emergence of Elopomorphs to another environment where its new location was stabilised. This change in the genomic environment of the Tshrb gene may alter the control of its expression, notably through epigenetic modifications and thus its response to environmental factors [44,45].

Like vertebrate Tshr, teleost Tshra is encoded by 10 exons. Nine of them encode the leucine-rich repeat (LRR) domain and, the large 10<sup>th</sup> exon encodes the transmembrane domain together with the carboxy-terminal cytosolic tail (Fig 6 and Fig. S3). This structure is conserved in eel Tshrb. In contrast, the domains encoded by the 10<sup>th</sup> exon appeared to be split into two exons in Tshrb from more derived teleosts (Fig. S3). Furthermore, the intracellular domain which usually exhibits signalization and internalization properties appeared to be shorter in TSHRb in these teleosts. However, alternative or additional exons could have been missed in our tentative sequencepredictions. Cloning of full length Tshrb cDNA in these teleost species could validate shortening of the cytosolic tail.

In Atlantic salmon, three Tshr sequences could be reconstructed from genomic data (Table S2). Phylogenetic analysis (Fig. 4) shows that two of them branch out together with the two characterized Amago receptors [46] and are of the a-type as previously shown [21]. These two a-type receptor genes likely result from the salmonid-specific genome tetraploidization event (4R) [47]. A third, partial Atlantic salmon Tshr sequence identified here appears to be of the b-type indicating that salmonids are likely to have both types of TSHR.

A single Tshr (Tshra) is present in the genome of tetraodontiformes (e.g. fugu, pufferfish). The loss of the duplicated Tshrb in this teleost group is in agreement with the fact that they possess only one TSH. In contrast, Tshrb was also absent in ostariophysians (zebrafish, Mexican tetra), while they possess two potential TSHs. This indicates at least two independent losses of Tshrbduring the radiation of teleosts. As with coelacanth or elephant shark TSH and TSH2, ostariophysian TSH and TSH3 might act through a unique TSHR receptor.

### TSHRa and -b sequences present specific signatures

The TSHR sequence alignment showed high conservation features throughout the vertebrate evolution (Fig 6, Fig. S3). As compared with FSHR and LHR sequences, both TSHR types present a specific long hinge region, delimited by two conserved cysteine boxes (cb2 and cb3) connecting the extracellular domain to the transmembrane domain. The fact that most of these TSHR structural features were maintained in TSHRb indicates that it is subjected to similar functional constraints.

In both types of TSHR, two potential glycosylation sites were conserved, one close to the LRR2 region (N88 of the alignment in Fig. S3) and the other at the end of LRR6 (N210), the latter being



**Figure 7. Tissue distribution profile of** *Tsh/, Tsh/3, Tshra* **and** *Tshrb* **mRNA in the eel.** Tissue distribution was analysed by RT-qPCR on RNA extracted from various tissues in the immature female European eel. Owing to the different nature of the tissues, transcript levels were normalized using total tissue RNA content: pituitary (Pit), thyroid follicles (TF), olfactory bulb (OB), mes-/diencephalon (Mes/), telencephalon (Tel), cerebellum (Cer), medulla oblongata (Med), eyes, liver, intestine (Int), muscle (Musc), adipose tissue (AT), gills, and ovary (Ov). Transcript levels of *Tsh*  $\beta$  subunit and of *Tshr* were normalized to the level in the pituitary and in the thyroid follicles, respectively and are expressed as arbitrary units. Results are represented as mean values  $\pm$  SEM (n = 8). doi:10.1371/journal.pone.0111361.g007

common to all glycoprotein hormone receptor types. The Nglycosylation site at the end of the cysteine box 2 (N324) was also conserved in both types of TSHR (except for the medaka TSHRb). The N-glycosylation site found in tetrapod sequences at the end of the LRR5 (N188) is conserved in lobe-finned fish and cartilaginous fish but is absent in ray-finned fish. Except in the eel, teleost TSHRb have additional potential N-glycosylation sites within the hinge region. They also differ from all other TSHR by lacking a conserved negative charge at position 262 at the start of the LRR9. A low amino acid conservation was observed within the hinge region for the TSHRb compared with the other TSHR (Fig. S3). In addition, one key cysteine of the cysteine-box 3 that was demonstrated to be involved in the disulfide bonding that gives its conformation to the hinge region is lost in cod. The THSRb hinge region lacks the common TSHR signature LKNPQ. The highly conserved tyrosine sulfation motif site [Y-(DE)-Y] within the hinge region involved in hormone recognition and signal transduction [48] that is still present in the eel is not conserved in more derived teleosts. In addition, the conserved glutamic acid (E) at position 446, which is a key determinant for the activation of the receptor [49], was switched to an alanine. Whether these differences in structure impact on binding specificity and on the signaling pathway will have to be determined.

TSHRb diverged at many points after the emergence of the eel as reflected by longer phylogenetic branch lengths (3.1 times that of TSHRa; cf Fig. 4 and Fig. S4), demonstrating an accelerated rate of evolution. Such a change in evolution rate is likely related to the duplication event with one gene keeping its original features (the type-a receptor) allowing the other to acquire new specificities in spatial or temporal control of its expression and in binding characteristics of its encoded protein [15,50]. Studies of functionality of the duplicated TSH and TSHR should be addressed in the future by developing recombinant hormone and receptors for various teleost species. Such investigations are required to characterize the binding selectivity of the two TSHR and to determine whether they activate the same signaling pathway.

#### A dual TSH system in the European eel

The tissue distribution analysis by qPCR of the two duplicated  $Tsh\beta$  transcripts in the immature female eel showed that the classical  $Tsh\beta$  subunit was exclusively expressed in the pituitary (Fig. 7). A low expression of  $Tsh\beta3$  could also be detected in the pituitary. Further in situ hybridization studies will be needed to pin-point if both duplicated  $Tsh\beta$  are expressed by the same pituitary cells. In addition,  $Tsh\beta3$  was highly expressed in the ovary and was detected in adipose tissue, gills, brain structures and eye. This is the first report of a comparative tissue distribution of the two  $Tsh\beta$  subunits in teleosts. It clearly shows that the two  $\beta$ subunits have a differential tissue expression, which may represent one of the evolutionary drives leading to the conservation of the duplicated hormone. In stickleback,  $Tsh\beta$  and  $Tsh\beta3$  expression was compared only in the pituitary where both are expressed but  $Tsh\beta\beta$  showed differential transcriptional regulation according to the ecotype [18]. Unlike in the eel, the classical  $Tsh\beta$  subunit was shown to be expressed not only in the pituitary but also in the gonads of the fathead minnow [51] and the grouper [52]. EST



**Figure 8.** *Tshf* and *Tshr* gene evolution. *Tsh* $\beta$  and *Tsh* $\beta$ 2 (left panel) were generated by duplication of a *preTsh* $\beta$  gene through the 2R before the emergence of Gnathostomes. *Tsh* $\beta$ 2 was lost (red crossed box) in tetrapods and in ray-finned fish before the emergence of the gar. *Tsh* $\beta$ 3 was generated in teleosts by duplication of *Tsh* $\beta$  through the 3R. Most teleost groups have kept *Tsh* $\beta$ 3. Only one *Tshr* (right panel) is found in vertebrates except in teleosts where a second gene was generated through the 3R. It was translocated to a new genomic environment after the emergence of the eel. Several groups of teleosts have lost this second *Tshr*. doi:10.1371/journal.pone.0111361.g008

data mining indicates that  $Tsh\beta$  transcripts are expressed in the brain of several teleost species (zebrafish - EH470445.1 and goldfish - DY231942; tilapia - GR616157.1; medaka -DK0206541) unlike the eel. In particular,  $Tsh\beta$  transcripts are found in the *saccus vasculosus*, at the base of the hypothalamus in salmon and may be involved as a photoperiodic signal transducer [53]. It is too early to draw conclusions about the absence of significant expression of the classical TSH in other tissues than the pituitary in the eel. Whether it is linked to its basal phylogenetic position relative to the duplication event, the physiological stage of the silver eel, or species specificity in the respective functions of the two TSHs will have to be further explored.

 $Tsh\beta\beta$  was identified among transcripts isolated from liver in the adult Antarctic toothfish (FE210400.1) and from the ovary in the yellow perch (GO658547.1) indicating that the expression of this gene in these species as well as in the eel, is not restricted to the pituitary gland.

In the eel, as with the two  $Tsh\beta$  subunits, the duplicated Tshr showed a differential tissue distribution pattern. The thyroid follicles showed a high expression of Tshra while a low level of Tshrb transcript could be detected (Fig.7). This suggests that Tshra would mediate the classically described thyrotropic endocrine

function of TSH. This is in agreement with the conserved synteny and sequence of teleost *Tshra* as compared with tetrapod *Tshr*. Together with the major expression of the classical *Tshβ* in the eel pituitary, this allowed us to infer that endocrine control of thyroid function via the classic TSH produced by the pituitary and classic TSHR expressed by the thyroid, is conserved through vertebrate evolution. Both *Tshra* and *Tshrb* are expressed in eel non-thyroid tissues with specific distribution. *Tshra* is expressed in the eye while *Tshrb* is mainly expressed in the pituitary and ovary and also detected in different parts of the brain and adipose tissue as well as some other peripheral tissues (Fig. 7). The similarity of expression profiles between *Tshβ3* might act as a paracrine or autocrine factor of TSHRb.

Non-thyroidal expression was reported for TSHRa in other teleosts, notably in the gonads in catfish [54,55] and fathead minnow [51], two ostariophysian species that, in contrast to the eel, might possess only this type of receptor. *Tshra* was also found in the gonad of striped bass [56] and European sea bass [57], species which may possess both receptors, according to their phylogenetic position among teleosts. The only available information about the tissue distribution of the b-type receptor is restricted

to the truncated transcript of Tshrb in the sole, that showed similar expression patterns as classical Tshra [20]. It will be interesting to investigate what effect the translocation of Tshrb from one genomic region to another has had on control of its expression.

In mammals, in addition to the thyroid follicles, TSHR expression has been described in several tissues notably anterior pituitary, hypothalamus, ovary, testis, skin, immune cells and adipose tissue [58–61] but the specific roles in these non-thyroid tissues are not fully known. As compared to the mammalian single TSHR, the tissue distribution of the duplicated eel TSHR highlights a clear sub functionalization, with TSHRa involved in the thyroid control and TSHRb in various non-thyroidal functions.

### Conclusion

The present study revealed that two TSHs would have arisen from the second global genome duplication (2R) in early vertebrates (Fig. 8), concurrently with the two gonadotropins LH and FSH [14]. The duplicated  $Tsh\beta 2$  has been conserved in cartilaginous fish and in early lobe-finned fish, but would have been lost both in tetrapods and in early ray-finned fish. This loss of the second  $Tsh\beta$  may be related to the redundancy of two TSH, acting via a unique receptor, since the putative duplicated TSH receptor issued from the 2R would have been lost early in the vertebrate evolution (Fig. 8). A second chance for a TSH system doubling occurred in teleosts when a novel global genome duplication (3R) occurred in the lineage. Both duplicated  $Tsh\beta/$  $Tsh\beta3$  and duplicated Tshra/Tshrb are found in various extant teleosts, while  $Tsh\beta3$  and Tshrb may have been lost in some teleost groups. The eel provides a remarkable example of conservation of a duplicated TSH/TSHR system. The endocrine thyrotropic function in the eel would be essentially mediated by the classical TSH and TSHR, which are mainly expressed in the pituitary and thyroid, respectively. The comparison of the distribution pattern of the duplicated  $Tsh\beta3$  and the duplicated Tshrb shows a striking similarity that could confer a possible autocrine/paracrine role for this couple in several non-thyroidal tissues in the eel. However, the higher evolutionary rate observed in teleosts for the duplicated  $Tsh\beta3$  and Tshrb suggests that their functions may not be fully stabilized yet. These advances in the evolutionary scenario of TSH and TSHR in vertebrates open new research avenues concerning the functional relationships between the two duplicated TSH and TSHR. Until now little is known about the TSH-TSHR system except in tetrapods. The structural characteristics of duplicated

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TSH $\beta$  in teleost have been well conserved suggesting it might be functional. Further investigations on ligand properties and receptor selectivity and activity are required to evaluate the biological importance of the duplication of the system TSH-TSHR and to infer evolutionary drives that contributed to the maintenance of the duplicated ligand –receptor system.

#### **Supporting Information**

Figure S1 TSH $\beta$  subunit-related sequences alignment. (PDF)

Figure S2 Reconstructed eel genomic regions flanking TSH $\beta$  (A) and TSHR (B) genes.

(PDF)

**Figure S3 TSHR-related sequence alignment.** (PDF)

Figure S4 Relative evolution rates between TSH $\beta$  and TSH $\beta$ 3 and between TSHR-a and -b sequences. (PDF)

Table S1 Database references for  $TSH\beta$  subunit-related sequences.

(PDF)

Table S2 Database references for TSHR-related sequences.

(PDF)

 Table S3 European eel primer sets for quantitative real-time PCR.

 (PDF)

Table S4 Database references for the genes in TSHβ and TSHR genomic regions. (PDF)

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### **Author Contributions**

Conceived and designed the experiments: GM BQ. Performed the experiments: GM BQ. Analyzed the data: GM SD JCT BQ. Contributed reagents/materials/analysis tools: GM SD BQ. Wrote the paper: GM SD JCT BQ.

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