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Striking antigen recognition diversity in the Atlantic salmon T-cell receptor α/δ locus

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Abstract

The complete TCR α/δ locus of Atlantic salmon (*Salmo salar*) has been characterized and annotated. In the 900 kb TCR α/δ locus, 292 Vα/δ segments and 123 Jα/δ segments were identified. Of these, 128 Vα/δ, 113 Jα, and a Jδ segment appeared to be functional as they lacked frame shifts or stop codons. This represents the largest repertoire of Vα/δ and Jα segments of any organism to date. The 128 functional Vα/δ segments could be grouped into 29 subgroups based upon 70% nucleotide similarity. Expression data confirmed the usage of the diverse repertoire found at the genomic level. At least 99 Vα, 13 Vδ 86 Jα, 1 Jδ, and 2 Dδ segments were used in TCR α or δ transcription, and 652 unique genes were identified from a sample of 759 TCRα cDNA clones. Cumulatively, the genomic and expression data suggest that the Atlantic salmon T-cell receptor has enormous capacity to recognize a wide diversity of antigens.

Keywords: Atlantic salmon; T-cell receptor α/δ locus; Antigen recognition; Gene rearrangement; Adaptive immunity

1. Introduction

Jawed fish and mammals are similar in their defense mechanisms against pathogenic infection as they both rely on innate and adaptive immunity. It is believed that the adaptive immune system arose in the gnathostome ancestor about 500 million years ago [1–3]. A fundamental component of the adaptive immune system is the T-cell, which has been considered the ancestral lymphocyte [4,5].

The T-cell has a membrane-bound receptor, which is responsible for antigen recognition and is composed of four distinct polypeptide chains (α, β, γ, and δ) There are two types of T-cell populations based upon their receptor heterodimers (α/β and γ/δ). The polypeptide chains are derived from variable (V), joining (J), diversity (D), and constant (C) gene segments. These segments are randomly selected from the germ-line gene pool by a recombination mechanism to generate a wide diversity for antigen recognition [6]. For mammals and birds, clusters of Vα, Jα,

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and Cα gene segments are orientated in the same direction, with the T-cell receptor (TCR) δ locus embedded between the Vα and Jα segments [7–9]. However, it has been reported that the V segments of teleost TCR α/δ locus are followed by Cα and Jα gene segments, which are in an inverted orientation to the V segments [10–12].

It is thought that fish adaptive immunity is intrinsically less efficient due to its evolutionary status and poikilothermic nature [13]. Furthermore, selection of specific mutants generated by hypermutation requires specific cell types and highly evolved specific lymphoid organs, which are yet to be identified in teleosts. Nurse sharks possibly possess anatomic tissue where affinity maturation could occur [14] and indeed affinity maturation has been demonstrated for an Ig isotype, IgNAR [15]. In addition, general studies have shown that fish antigen recognition molecules (i.e. Ig superfamily, MHC, and T-cell receptor) vary in their repertoire at the genomic level from species to species [16–21].

Partial TCR α sequence data have been reported for various teleosts including rainbow trout [1], catfish [22], zebrafish [12,23–25], Atlantic cod [26], Japanese flounder [27], bicolor damselfish [28], Atlantic salmon [29], carp [30], turbot [31], and puffer fish [10,11]; however, the extent of TCR diversity in fishes has not been well documented. Two fully sequenced species of puffer fish (*Takifugu rubripes* and *Tetraodon nigroviridis)* have little diversity in TCR α/δ locus [10,11], and although the zebrafish genome is sequenced, the complete TCR locus α/δ is yet to be completely characterized. The objective of this project is to learn the functionality of Atlantic salmon's adaptive immunity and to identify the extent of its possible recombinational diversity by completely characterizing its TCR α/δ locus. This will provide important key information for the understanding of the fish adaptive immune system and evolutionary relationships of the TCR to other vertebrates.

2. Materials and methods

2.1. Screenings and sequencing of Atlantic salmon TCR α/δ locus

The Atlantic salmon CHORI-214 bacterial artificial chromosome (BAC) library, constructed from a Norwegian aquaculture strain male, was obtained from BACPAC Resources, Children's Hospital Oakland Research Institute (CHORI) [32]. Six BAC library filters were hybridized with a probe encoding the TCR α constant region. The TCR probe was generated with the following primer set: αC-F and αC-R and 5′-end labeled with γ^{32}P using T4 polynucleotide kinase (Invitrogen, Inc., Carlsbad, CA). Labeled probes were added to prehybridized BAC filters, which were prehybridized at 65 °C for 4 h (5 × SSC, 5 × Denhardt's, 0.1% SDS). Hybridization was carried out overnight at 65 °C. Three washes were performed, each for 30 min at 50 °C, the first consisting of 2 × SSC and 0.1% SDS, and the second and third consisting of 1 × SSC and 0.1% SDS. Filters were visualized using BioMax film (Kodak). Further, BAC clones were identified by designing two 70mer oligo probes based on BAC sequence data: TCR2-1 and TCR2-2 (Integrated DNA Technologies). Probe labeling and hybridization were conducted as described above. BAC clones were chosen based on the physical BAC fingerprint map for Atlantic salmon, which is publicly available on the Internet Contig Explorer (iCE) version 3.4 [33]. BAC shotgun libraries were constructed and sequenced on an ABI 3700 DNA sequencer, resulting in 7–10 fold sequence coverage for each BAC clone, each of which was assembled using PHRED [34], PHRAP [35], and Consed [36].

BAC clone sequence data were annotated using BLAST homology searches and EMBOSS programs package (http://emboss.sourceforge.net/). Alignment of cDNA sequences against genomic sequences was done by Sequin [37], which was obtained from NCBI.

Dotter [38] was used to compare BAC sequences and to identify duplicated regions. A 50 kb duplicated region in BAC 36P14 was validated by PCR analysis. A PCR primer set was designed in the intron between V17 and V18 genes: V17–18-F and V17–18-R. Six individual Atlantic salmons were used for the PCR analysis, and BAC 36P14 and 249L01 were used as controls. PCR was performed with an initial denaturation step of 2 min at 95 °C and then 20 cycles of the following: 30 s of denaturation at 95 °C, 30 s of annealing at 55 °C, and 1 min of extension at 72 °C. PCR products were separated by electrophoresis on a 1.5% agarose gel.

2.2. Fluorescent in situ hybridization (FISH)

BAC 249L01 was used as a probe for FISH on Atlantic salmon chromosomes. FISH and identification of chromosomes were conducted with methods described previously by Phillips et al. [39].

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2.3. mRNA expression in Atlantic salmon tissues by RT-PCR

Total RNA was extracted from healthy Atlantic salmon tissues (head kidney, muscle, skin, gut, gill, spleen, brain, heart, gonad, liver, eye, pyloric caeca, and thymus) using TRIzol reagent (Amersham Pharmacia Biosciences). Purified total RNA (5 μg) was reverse transcribed with SUPERSCRIPT^{TM} II (Invitrogen) and an 18-nucleotide oligo d(T) primer as described in the manufacture's protocol. The PCR primer sets used were αRT-F and αRT-R for TCR α, δRT-F and δRT-R for TCR δ, βRT-F and βRT-R for TCR β, γ1RT-F and γ1RT-R for TCR γ1, γ2RT-F and γ2RT-R for TCR γ2, and ubiqRT-F and ubiqRT-R for ubiquitin as an internal control. PCR was performed with an initial denaturation step of 2 min at 95 °C and then 20 cycles as follows: 30 s of denaturation at 95 °C, 30 s of annealing at 55 °C, and 1 min of extension at 72 °C. PCR products were electrophoresed on a 2.0% agarose gel.

2.4. Cloning and sequencing of TCR α/δ cDNA

Total RNA was extracted and reverse transcribed from Atlantic salmon thymus as described above. Ninety-seven forward primers were designed for functional V segments found in the TCR α and δ locus. A reverse primer and a nested reverse primer were designed in the constant region of the TCR α or δ locus. PCR was performed with an initial denaturation of 2 min at 95 °C and then 30 cycles were run at 30 s of denaturation at 95 °C, 30 s of annealing at 55 °C, and 1 min of extension at 72 °C. PCR products were cloned into pCR2.1 vector (TA Cloning Kit, Invitrogen) with the manufacturer's protocol. Each positive PCR product was sequenced as described above, and the V and J segments were identified for each TCR cDNA clone.

The PCR primers and oligo probes used in this study are shown in Supplemental Table 1.

3. Results

3.1. Sequence and annotation of the TCR α/δ locus

BAC 249L01 hybridized to one locus on the Atlantic salmon chromosome pair 14 by FISH analysis (Supplemental Fig. 1). This result suggests that there is a single TCR α/δ locus in Atlantic salmon. Six salmon genomic BAC clones containing the TCR α/δ locus were identified and sequenced: 508L05 (203,865 bp), 251P16 (182,732 bp), 15J19 (213,804 bp), 39N03 (189,019 bp), 36P14 (182,614 bp), 249L01 (224,724 bp), and 31E09 (203,216 bp) (Fig. 1). In this 900 kbp region, we identified a constant α region, a constant δ region, 123 J segments, 292 V segments, and 3 δ D segments. Furthermore, 60 additional V segments arose from a tandem duplication (BAC 36P14 and 508L05). Flanking the TCR α/δ locus, cytochrome P450 family 7 subfamily B polypeptide 1 (CYP7B1) was found in BAC 598L05 and a homolog of nonsense-mediated mRNA decay protein (SMG-7), laminin γ 1 (Lamc1), and nicotinamide nucleotide adenylyltransferase 2 (NMNAT2) genes were found in clone 31E09 (Fig. 2). Dot-matrix analysis revealed that BAC 249L01 and 36P14 overlapped and that BAC 36P14 contained a duplicate region of BAC 249L01 (Fig. 3A). Confirmation of this polymorphic 50 kb duplicated region was carried out using PCR analysis. A primer set was designed to only amplify a single product in BAC 249L01, whereas two products would be amplified if the duplicate region existed, as found in BAC 36P14. These two products would differ in size, as the product from the duplicate region would have a 30 bp deletion. PCR analysis of the six individuals resulted in one individual having only one band as per BAC 249L01 control PCR, while the other five individuals had two bands as found with BAC 36P14 (Fig. 3B). This suggests that there are at least two different TCR α/δ alleles. BAC 508L05 also contained a partial double-duplication region as shown in Fig. 1.

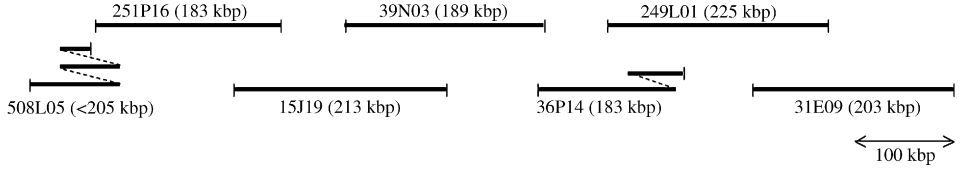

Fig. 1. Physical map of the Atlantic salmon TCR α/δ locus. Overlap of BACs used in this study is summarized. The approximate sizes of the inserts are indicated in parentheses.

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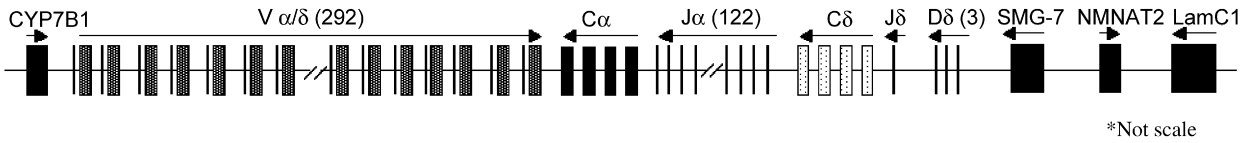

Fig. 2. Genomic organization of the Atlantic salmon TCR α/δ locus (not to scale). TCR α/δ genes, cytochrome P450 family 7 subfamily B polypeptide 1 (CYP7B1), nonsense-mediated mRNA decay protein (SMG-7), laminin γ 1 (Lamc1), and nicotinamide nucleotide adenylyltransferase 2 (NMNAT2) genes are represented by boxes. Exons for the CYP7B1, LamC1, NMNAT2, and SMG-7 genes are not shown. The numbers of each segment are indicated in parentheses. Arrows indicated their transcriptional orientations.

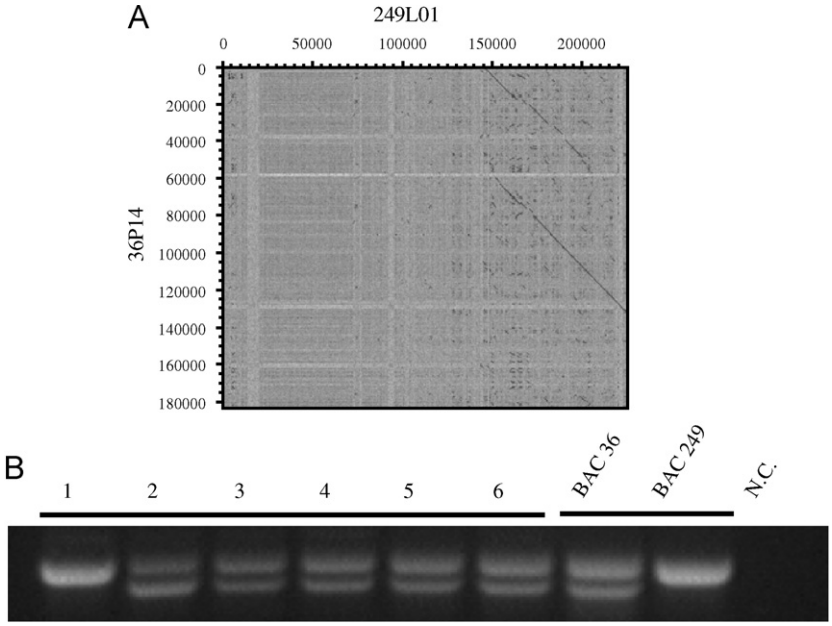

Fig. 3. (A) Dot-matrix analysis of BAC 249L01 sequence (horizontal) versus BAC 36P14 sequence (vertical). The result shows that these two BAC clones overlap and the BAC 36P14 contains a duplication in the region from 150 to 200 kbp of BAC 249L01. (B) Validation of the 50 kb polymorphism in the TCR α V region. Lanes 1–6: different Atlantic salmon individuals: BAC 36: BAC 36P14; BAC 249: BAC 249L01; N.C.: negative control (TE).

3.2. Constant regions

From the genomic sequence data, we identified a constant α and a constant δ region, both of which contain four exons and three introns. The constant α region is identical to that of a previously reported Atlantic salmon constant α region by Hordvik et al. [29] (GenBank, accession number AY552003, except for the 5′-prime region). The constant gene consists of an immunoglobulin constant region, connecting peptide, transmembrane, and cytoplasmic domains similar to other vertebrates.

3.3. J segments

A total of 123 J segments were found in the Atlantic salmon TCR α/δ locus (Fig. 2). Located between the constant α and constant δ regions were 122 Jα segments and a single functional Jδ segment was located between Dδ segments and the constant δ region. All J segments and constant α/δ genes are translated in the same direction. Of the 122 Jα segments, nine are pseudogenes as they have stop codons or frame shifts in their open reading frame (ORF) (Table 1). The single Jδ segment was deemed functional based upon its intact ORF. In the J segments, a core sequence of phenylalanine–glycine–X–glycine–threonine (FGXGT) was highly conserved. The gene names, functionality, genomic sequences, and deduced amino acid sequences of all J segments are shown in Supplemental Table 2. J segments are flanked by a consensus recombination signal sequence (RSS) and a splice site. The RSSs have the typical sequence structure of a nonamer (RGTTTTTGT) and a heptamer (YAYTGTG), which

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Table 1
Comparison of the total number of segments that constitute the TCR α/δ locus for Atlantic salmon versus other species

	Total no. of V segments	Functional V segments	V family	Total no. of J segments	Functional J segments	Total no. of D segments	Constant region
A. Salmon	292	128	62	122 Jα, 1 Jδ	113 Jα, 1 Jδ	3	1α, 1δ
Human	57 (49α, 5α/δ, 3δ)	46 (38α, 5α/δ, 3δ)	41	61 Jα, 4 Jδ	50 Jα, 4 Jδ	3	1α, 1δ
Mouse	98 (91α, 7α/δ, 6δ)	89 (91α, 7α/δ, 5δ)	23	61 Jα, 2 Jδ	38 Jα, 2 Jδ	2	1α, 1δ
Chicken	70 (40α, 30δ)	NA	3 (2α, 1δ)	25 Jα, 2 Jδ	25 Jα, 2 Jδ	2	1α, 1δ
Zebrafish	>148	>89	87	>71 Jα, 2 Jδ	>71 Jα, 2 Jδ	2	1α, 1δ
Tetraodon	13	13	6	12 Jα, 2 Jδ	12 Jα, 2 Jδ	4	1α, 1δ

are separated by the 12 bp spacer. The highly conserved consensus 6 bp splice site (GTAAGT) is located at the 3′-end of the J segment's coding region.

3.4. V segments

The TCR α/δ locus contained 292 unique V segments. In addition, 26 V segments in BAC 36P14 and 34 V segments in BAC 508L05 appear to have arisen from tandem duplications. Of the 352 TCRα V genes, 128 unique V segments are potentially functional. The remaining 164 V segments are considered pseudogenes as they contain stop codons or frame shifts in their ORF (Table 1). Based upon a 70% nucleotide sequence similarity, all Vα or δ segments could be grouped into 62 different V subgroups. When this analysis was constrained to just the functional V segments, they segregated into 29 subgroups. The amino acid sequences for the TCR V segments can be divided into a framework region (FR) and a complementary determining region (CDR). All of the functional V segments have canonically accepted sequence structures: the conserved cysteine residues involved in the intradomain disulfide bond of the V domain, the conserved motif of WYXQ in the FR2, and the YYCA motif in the FR3 regions. We also found RSSs located in the 3′- ends of each V segment coding region. RSSs consisted of a heptamer (CACAWGTG) and nonamer (ACAAAAAMC) separated by 23 or 22 bp spacers. The gene names, functionality, genomic sequences, and deduced amino acid sequences of all V segments are shown in Supplemental Table 2.

3.5. Dδ segments

Identification of the Dδ segments could not be determined based upon sequence alignment with other species, as the sequence of this segment is typically short and highly diverse. By identifying the diverse region sequence in Atlantic salmon TCR δ EST clones, we were able to align this sequence to that of the genomic sequence. Location of this alignment and determination of the 5′-flanking 12 bp RSS and the 3′-flanking 23 bp RSS led to the identification of 3 Dδ segments. The gene names and sequences of 3 Dδ segments are shown in Supplemental Table 2.

3.6. TCR α and δ EST clones

We found 82 Vα and 3 Vδ EST clones in the public database. There are only two redundant clones in a total of 85 EST clones. A majority of the Atlantic salmon TCR α and δ EST clones are found in thymus cDNA libraries (37 clones, 47.4%). Thirty-four different Vα and 32 Jα segments occurred in TCRα EST clones, and three different Vδ and one Jδ segment were found in the TCRδ EST clones.

3.7. Expression of Atlantic salmon TCR genes from various tissues

As a step toward evaluating function and localization of various Atlantic salmon TCR molecules, we assessed the expression of the TCR genes in various tissues by RT-PCR. It was found that TCR α, β, and γ1 mRNAs are expressed in the head kidney, gill, spleen, pyloric caeca, and thymus. TCR δ and γ1 mRNAs were also expressed in the liver, whereas TCR γ2 mRNA was primarily expressed in the gill and thymus (Fig. 4).

3.8. Isolation of TCR α/δ cDNA clones

In 759 sequenced clones, a total of 652 non-redundant TCRα cDNA sequences were identified.

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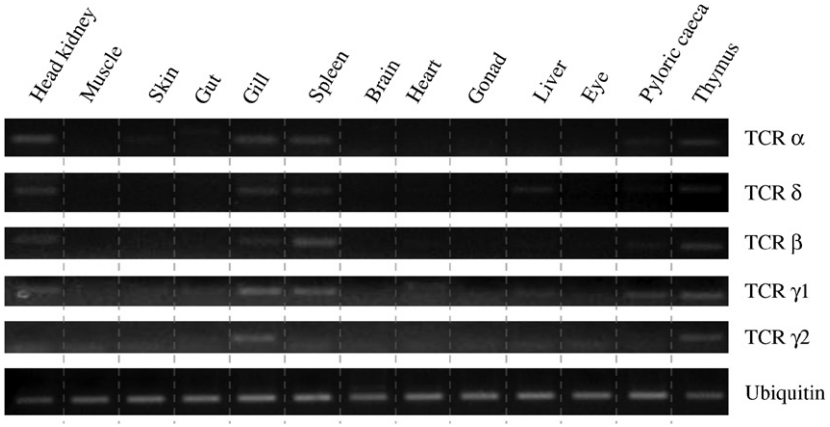

Fig. 4. RT-PCR analysis of TCR genes in various Atlantic salmon tissues. TCR α, δ, β, and γ1 mRNA were expressed in the head kidney, gill, spleen, pyloric caeca, and thymus. TCR δ and γ1 mRNA were also expressed in the liver, and TCR γ2 mRNA was expressed only in the gill and thymus. Ubiquitin is an internal control.

Of these, 586 clones (89.9%) were deemed potentially functional based upon their ORF. The remaining 66 clones (10.1%) are possibly non-productive rearrangements as they contain multiple frame shifts or stop codons in their sequence. For functional TCRα cDNA clones, the number of N-region nucleotide additions were 0–7 nucleotides. Sequence data of the TCRα cDNA clones indicated the expression of 89 functional and 3 non-functional Vα segments and 83 unique Jα segments. These 89 unique functional Vα segments represented 26 of the 29 V functional subgroups that were identified by the genomic data (Table 2).

Sequencing 45 TCRδ cDNA clones resulted in a total of 25 non-redundant clones being identified, of which 19 (76.0%) are functional based upon ORF sequence analysis. Non-productive rearrangements were detected in the remaining six TCRδ cDNA clones (24.0%). The number of N-region nucleotide additions were 0–19 nucleotides. For the 25 TCRδ cDNA clones that are presumed to be functional, 11 different V segments were utilized. We confirmed that these V segments were also used by TCR α cDNA clones. All of the TCR δ cDNA clones had the Jδ segment flanking the 5′-end of the constant δ gene. Although we identified 3 Dδ segments, one of the Dδ segments, which is located most distant from the constant δ region, was not represented in any of these 25 TCR δ cDNA clones (Table 2).

Table 2
Summary of TCR α/δ cDNA clones obtained from expression study

	TCR α		TCR δ	
Total no. of sequenced clones	759		45	
Total no. of unique clones	652	85.9%	25	55.6%
Functional	586	89.9%	19	76.0%
Non-functional due to				
Frameshift	63	9.7%	4	16.0%
Stop codon	2	0.3%	2	8.0%
Partial	1	0.2%	0	0
No. of V usage	92 Vα		11 Vδ	
No. of D usage	–		2 Dδ	
No. of J usage	83 Jδ		1 Jδ	
V–(D)–J combination	533		12	
N-region nucleotide addition	0–7 nucleotides		0–19 nucleotides	

4. Discussion

It has been previously stated that there is limited diversity of V segment usage for TCRα or a variety of teleosts including rainbow trout [1], catfish [22], zebrafish [25], Atlantic cod [26], Japanese flounder [27], bicolor damselfish [28], Atlantic salmon [29], carp [30], turbot [31], and puffer fish [10,11]. However, our results indicate that the Atlantic salmon TCR α/δ locus is highly diverse at the genomic level, as well as at the expression level.

Characterization of the complete locus is supported by the identification of CYP7B1 in the 5′-flanking region, and SMG-7, Lamc1, and NMNAT2 in the 3′-flanking region (Fig. 2). These genes and the TCR α/δ locus are also syntenic for

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zebrafish chromosome 2 and *Tetraodon* chromosome 15 (NMNAT2 gene was not found in *Tetraodon* chromosome 15). In mouse and human, the TCR α/δ locus is located on chromosome 14 and the SMG-7, NMNAT2, and Lamc1 loci are located on chromosome 1. These results suggest that a large group of genes around the TCR α/δ locus are syntenic in the common fish ancestral chromosome. However, there are significant differences in the organization of mammalian and fish chromosomes containing the TCR α/δ locus.

The Atlantic salmon TCR α/δ locus contains a total of 128 functional Vα/δ segments and 113 functional Jα segments (Fig. 2). This represents the largest number of such segments found to date in any species (Table 1). Insertion or deletion of nucleotides in the 163 pseudo Vα segments could potentially give rise to further diversity in the TCR. Furthermore, we found two alleles in the Atlantic salmon TCR α/δ locus. BAC 36P14 and 508L05 contain 60 V segments, which arose from a tandem duplication. These duplicated V segments were slightly different from the original V segments (93.7–100% nucleotide similarity). Thus, individuals possessing the duplicated allele (Fig. 3B) potentially have a 20.5% increase (60 duplicated V segments in a total of 292 V segments) in their V segment variability. The 128 functional Vα/δ segments could be grouped into 29 different V subgroups ($<70\%$ identical nucleotide), and expression was confirmed in 26 of the 29 V subgroups. It has been reported that the zebrafish can be grouped into 87 Vα families, although only 10 Vα families are reported to be expressed at relatively high levels [25]. In mammals, there are 41 Vα subgroups and 4 Vδ subgroups in human and 23 Vα subgroups and 6 Vδ subgroups in mouse (IMGT Repertoire, http://imgt.cines.fr). Thus, the teleost TCR α and δ V segments are potentially at least as diverse as those of mammals.

The genomic organization of Atlantic salmon TCR α/δ locus is similar to that of Japanese puffer fish [10], *Tetraodon* [11], and zebrafish [12] in that the major regions are in the same order and the V region is in an opposite coding orientation relative to all other regions (Cα, Jα, Cδ, Jδ, and Dδ) (Fig. 2). This suggests that this is a common feature in the teleost TCR genomic organization. Contrastingly, most V segments in the mammalian V region are in the same transcriptional orientation as all other regions and the region order differs from that of teleosts. Although all functional V, J, and D segments in Atlantic salmon have the canonically accepted RSS and splicing signal sequence in their flanking regions, their difference in transcriptional order and orientation to that of mammals means that they rely on a different mode of recombination to generate a functional TCR. Recombination of the Atlantic salmon TCR α/δ gene occurs by a DNA inversion and therefore does not delete gene segments between the two recombined gene segments. These regions may be retained in an inverted orientation and possibly generate greater diversity and flexibility for antigen recognition. Such an inversion recombination was frequently observed in the teleost Ig superfamily [40]. Surprisingly, and unlike mammals, a larger percent (48.9%) of the functional Atlantic salmon V segments use a rare 22 bp spacer in the RSS to generate V–J recombinants without the expected drop in the recombination efficiency [41,42].

The large diversity seen at the genomic level was further corroborated by the expression results. Based on our 652 unique TCR α/δ cDNA clones and publicly available data (NCBI database and [29]), at least 99 Vα (including 7 non-functional Vα segments), 13 Vδ, 86 Jα, and 1 Jδ are capable of being rearranged and transcribed functionally from the TCR α/δ germ-line repertoires identified. Thus, our expression data captured 71% and 79% of the total potentially functional V and J segments, respectively, and showed a very high level of unique transcripts. Screening of various tissue types for TCR expression by RT-PCR indicates that TCR genes are mainly expressed in the head kidney and thymus. However, δ and γ1 were also expressed in the liver, whereas γ2 was only expressed in the gill and thymus (Fig. 4). Moreover, most of TCR α and δ EST clones were found in the thymus cDNA library. These results suggest that the Atlantic salmon TCR α/δ locus has differential transcriptional regulation in a broader range of tissues than found in mammals. The two different chains making up the TCR heterodimer (i.e. α/β and δ/γ) were consistently coexpressed in each specific tissue type.

The TCR δ expression data showed the use of two of the three Dδ segments identified in the genomic sequence. TCR δ clones from RNA either utilized both δ segments, one of the δ segments, or lacked a Dδ segment altogether. In all cases, Dδ segments were flanked by N-regions (Fig. 5). Nucleotide deletions in the Dδ segments generated further diversity, which has also been seen in a *Tetraodon*

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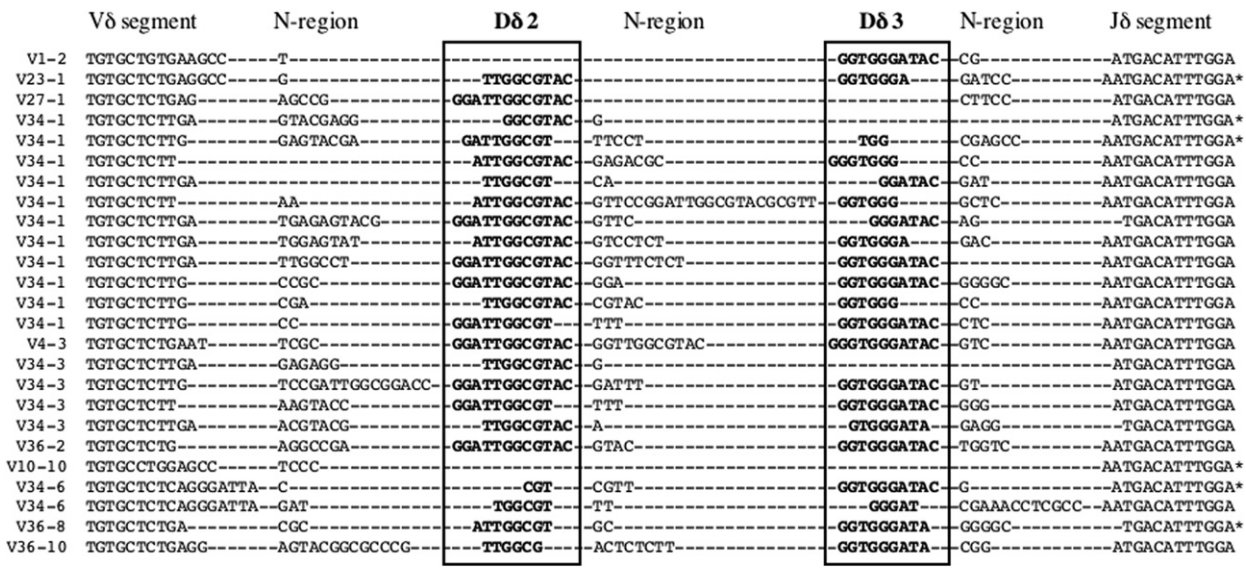

Fig. 5. Alignment of V–(D)–J junction for TCR δ cDNA clones obtained from the expression study. The V segment usage is listed to the left of sequences. Dδ segments are indicated by boxes. Out-of-frame clones are noted (*).

study [11]. Genomic data for both Atlantic salmon and *Tetraodon* identified a distal Dδ segment; however, in both cases its transcription product was not detected even though it possesses all the appropriate recombinational mechanisms. Further work is required to determine if this distal segment is utilized or not. Interestingly, the 13 V segments used to generate TCR δ cDNA clones were also found in the TCR α cDNA clones, demonstrating V segment bifunctionality. We cannot conclude that all V segments have this bifunctionality for both the α and δ TCRs, as the expression data are limited in this study, particularly for TCR δ clones.

There are several published and publicly available sequences for individual rainbow trout TCR α V and J segments. The calculated percent identities for both V and J segments were greater in inter-species than in intra-species comparisons. This indicates that the TCR α V and J segments may be conserved among salmonids. However, we could not find the same large diversified repertoires of TCR α/δ molecules in the sequence database of rainbow trout such as we have described here for Atlantic salmon.

TCRs recognize antigen peptides with their CDR3, which is composed of V segments, J segments, D segments (in β and δ TCR), and a non-template (N)-region. Flexibility for antigen recognition is determined by recombinational events in the V and J segments as well as insertion or deletion of nucleotides in the N-region [43]. Analysis of the CDR3 nucleotide and amino acid sequence in the TCR α cDNA clones indicates that Atlantic salmon, like mammals, modify the N-region (Fig. 6), further increasing their capacity to recognize a wide variety of antigens.

Based upon available literature, we identified the total number of V and J segments that have been documented in genomic data for human, mouse, zebrafish, and *Tetraodon*. V segments were then further categorized into V subgroups based upon 70% nucleotide similarity (Table 1). Atlantic salmon seem to have the greatest number of functional V segments compared with all other organisms characterized to date as well as a surprisingly large number of pseudogenes. However, when the V segments are grouped into subgroups, Atlantic salmon is more in line with human and mouse. Zebrafish stands out with the greatest number of diverse V subgroups [25]. The human TCRs use fewer numbers of V segments and larger subgroup diversity, whereas Atlantic salmon rely upon a large number of similar V segments to generate their V segment diversity. The number of both V and J segments in *Tetraodon* is significantly lower than those of the other species. The variety of J segments in Atlantic salmon seems to be twice that seen in human and mouse.

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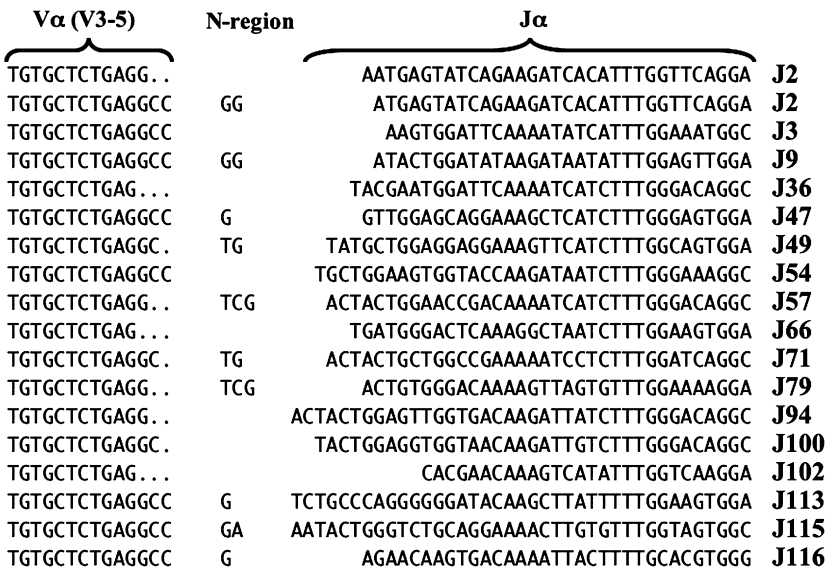

Fig. 6. Alignment of the Atlantic salmon TCR α V–J junction region. The V3–5 segment was used for all clones shown. Dots indicate truncation in the V3–5 segment. J segments with known genomic location and sequence are indicated on the right.

This study reveals that the Atlantic salmon TCR α/δ genes have enormous diversity in their capacity for antigen recognition due to the large numbers of V and J segments, allelic polymorphisms, extensive recombinational possibilities, and N-region diversity. The expression data identified 68% and 79% of the potentially functional V and J segments found at the genomic level. It has been reported that the recombination features of TCR molecules in fish most generally lead to diversity of the expressed TCRs, but that the initial repertoire of V and J segments seems to be much less diversified in fish than in mammals [1,10,11,22,26–31]. However, Atlantic salmon clearly has one of the largest repertoires known of any vertebrate, suggesting that adaptive immunity may play a significant role in the health of this fish.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dci.2007.05.002.

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