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A second immunoglobulin light chain isotype in the rainbow trout

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Abstract A novel immunoglobulin (Ig) light chain isotype, termed IgL2, has been isolated from trout lymphoid tissues both by reverse transcription – polymerase chain reaction (PCR) and screening of cDNA libraries. The CL domain of the new isotype shares only 29% residues with a recently cloned trout IgL isotype, termed IgL1, which has some similarities to Cκ and Cλ isotype domains of several vertebrate species. Using anchored PCR, a *VL* element rearranged to *CL2* was isolated. It is a member of a new VL family (VL2) of which four members were sequenced. These differ in the sequence of CDR1 and CDR2 but are remarkably similar in CDR3, i. e., at the junction between *VL* and *JL* segments. *VL* elements are rearranged to novel *JL* elements which differ from those described for *VL1-CL1* rearrangements. Two cDNA clones contained *JL-CL2* segments but no *VL* segments. The *JL* segments were preceded by typical rearrangements signal sequences [*RSS*, nonamer-23 base pair (bp) spacer-heptamer]. Further upstream of *RSS* were located two to three near identical 53 bp repeats, each of which included a 16 bp sequence similar to *KI* and *KII* sequences located at similar places in human and mouse *Jk1* genes. These sequences are believed to act as binding sites for the protein KLP, which could be a transcriptional factor involved in the synthesis of germline *Jk* transcripts. Their phylogenic conservation in vertebrates suggests that they have an important role in B-cell differentiation. Remarkably, an RNA species of about 0.7 kilobase is the predominant *IgL* mRNA in trout spleen and coincides in size with *JLCL2* transcripts. Genomic DNA blot analysis indicates that the trout *L2* locus has a cluster-like organization similar to the trout *L1* locus and the *IgL* locus of several teleost fish. A phylogenic analysis of VL2 and CL2 corroborates their low similarity to other vertebrate IgL chains and suggests an ancient diversification of the *IgL* locus.

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Introduction

Immunoglobulins (Ig) are the effector molecules of specific humoral immunity in all jawed vertebrates. Teleost fish possess a single class of tetrameric Ig, each subunit comprising two pairs of covalently-linked heavy-light (H-L) chain dimers. Recent structural and phylogenic analysis of the H chains of several species of teleost fish confirmed that they are clearly related to the μ chain isotypes of higher vertebrates and to the IgH chains of elasmobranch (Andersson and Matsunaga 1993). Earlier studies have shown that there are structural and antigenic L chain variants in several teleost species. The channel catfish (*Ictalurus punctatus)* has two distinct (F and G) L chain isotypes, which differ in relative molecular mass (M_r) and antigenicity (Lobb et al. 1984). Catfish genes for the L chain G isotype have recently been cloned, but they cannot be readily classified as mammalian-like κ or λ isotypes (Ghaffari and Lobb 1993). These catfish *IgL* genes have a complex genomic organization reminiscent of the clustered organization of the elasmobranch *IgL* genes (Shamblott and Litman 1989). Two L chain variants have been described in the Atlantic salmon (*Salmo salar*; Harvarstein et al. 1988) and the rainbow trout (*Oncorhynchus mykiss*; Sànchez et al. 1989). The two L chain variants in the trout can be distinguished by monoclonal antibodies and are produced by all individual trout studied, as well as by other salmonid fish, like the brown trout (*Salmo trutta*), the coho salmon (*Oncorhynchus kitutch*), and the chinook salmon (*Oncorhynchus tschwytscha*; Sànchez and Dominguez 1991). The

Atlantic cod (*Gadus morhua*) L chain-encoding genes and the gene for one isotype of the rainbow trout L chains have been recently characterized (Daggfeldt et al. 1993). The rainbow trout L chains cannot be assigned to be κ or λ-like and the *IgL* locus has a clustered organization of *VL* and *CL* segments, similar to the findings in catfish (Ghaffari and Lobb 1993). The aim of this study was to characterize a second rainbow trout L chain isotype (IgL2) which is clearly different from the recently described trout IgL1 chain (Daggfeldt et al. 1993).

Materials and methods

Isolation of a polymerase chain reaction (PCR)-derived cDNA clone encoding a trout CL2 segment

First-strand cDNA was synthesized from total trout thymus RNA (see Discussion) using the oligonucleotide GACTCGAGTCGACATC-GAT_{17}, as primer (Frohman et al. 1988). RNA amplification was carried out in 30 µl reaction mixture containing about 1% of the cDNA/RNA hybrid, the above 3′ end primer and a 32-fold degenerated primer designed from a conserved stretch of amino acid residues located around the N-terminal cysteine of vertebrate Ig Cλ and TcR Cβ domains: [5′-GGGAATTCAAGGCYACMCTSGTGTGYYTG, where Y = (T/C), M = (A/C), and S = (G/C)] (Fellah et al. 1993). The amplified DNA [about 600 base pairs (bp)] was purified (Genclean; Bio 101, Vista, CA) and cloned in the *Eco* RI/*Sal* I sites of pBluescript (Stratagene, La Jolla, CA). One of the cDNA clones (*CL2*) coded for the constant region of a putative trout *IgL* gene (see Results).

Construction and screening of a trout spleen cell cDNA library

Total RNA was extracted from the pooled spleen cells of four non-immunized trouts, cDNA was synthesized using a commercial kit (Pharmacia, Uppsala, Sweden) and a cDNA library was constructed in the λZAP II vector (Stratagene), following the manufacturer's protocol. The library was screened with a 365 bp ^{32}P-labeled random-primed probe obtained by digestion of clone *CL2* with *Eco* RI/*Eco* RV. Positive clones were purified and excised in vivo with the M13 VCS helper phage as suggested by the manufacturer (Stratagene).

Isolation of trout VL2 segments by anchored PCR

Total cytoplasmic RNA from the spleen of a single non-immunized trout was used to construct a *VL2* library by anchored PCR (5′ Amplifinder Race Protocol; Clontech Palo Alto, CA). Briefly, first-strand cDNA was synthesized using an antisense-specific primer (5′-ATTTGACTGGCCAGACAC) and reverse transcriptase (Superscript Reverse Transcriptase; Gibco/BRL, Eggenstein, Germany). The RNA template was removed and the first cDNA strand purified. A single-stranded anchor oligonucleotide was ligated to the cDNA 3′ end using T4 RNA ligase. A portion of this anchored cDNA was then used as a template for PCR amplification using the anchor manufacturer's 5′ primer and a nested gene-specific 3′ primer (5′-GCGAATTCCGTC-GACGAGGATGGCAAGACAG). The amplification product was then cloned in the *Eco* RI/*Sal* I sites of the pBluescript SK^+ vector (Stratagene).

DNA sequencing

The PCR and recombinant clones were sequenced independently in both strands by the dideoxy method (Sanger et al. 1977) using universal and specific primers.

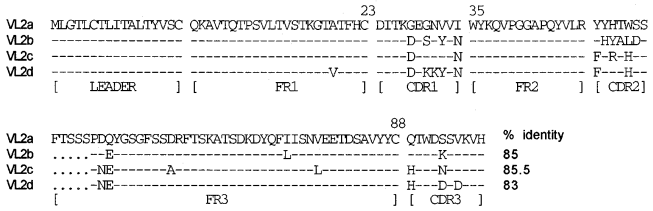


Fig. 1 Alignments of the amino acid sequences of four rainbow trout *VL2* segments belonging to the same family. Residues identical to the top sequence are denoted by *hyphens*, and spaces introduced to optimize similarity between sequences are indicated by *dots*. The putative leader, FR, and CDR regions are defined following Kabat and co-workers (1991). The GenBank accession numbers of the corresponding nucleotidic sequences are U69987 (*VL2a*); U69988, (*VL2b*); U69989 (*VL2c*); U69990 (*VL2d*)

Northern blotting experiments

Total spleen, thymus, and brain RNA were denatured, fractionated on agarose-formaldehyde gels, transferred to nylon filter, and hybridized to a ^{32}P-labeled random-primed probe corresponding to the full-length *VL2-CL2* insert of clone 15 selected from the cDNA library (see Results). The northern blot was washed and autoradiographed at –80 °C for 1–3 days.

Southern blotting experiments

DNA was extracted from trout erythrocytes, digested with *Hin*dIII, *Bam* HI, *Eco* RI, *Pst* I or *Knp* (Boehringer Mannheim, Mannheim, Germany), electrophoresed (10 µg/lane) through 0.8% agarose and transferred to Hybond-N+ filters (Amersham, Les Ulis, France). Hybridization was performed for 16 h at 42 °C in a 5 × saline sodium phosphate-EDTA (SSPE) buffer; 5 × Denhardt's; 50% formamide; 0.1% sodium dodecyl sulfate (SDS) solution containing 100 µg/ml *E. coli* tRNA (Sigma, St. Louis, MO), using ^{32}P-labeled random-primed probes corresponding to the constant CL2 or variable VL2 domains of the L2 chain, isolated from clone 15. Filters were washed at 50–65 °C with, successively, 4, 2, 1 × SSPE buffer/0.1% SDS and autoradiographed at –80 °C for 1–3 days.

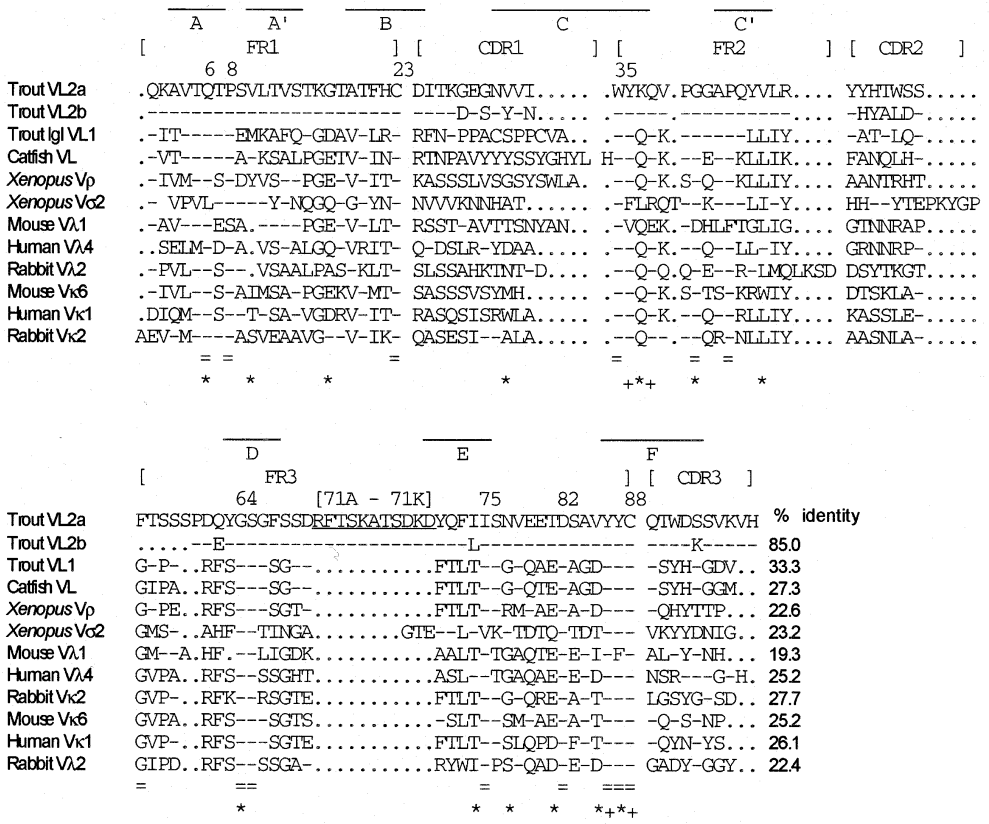
Construction of distance trees

The multiple alignment and graphical relationship were constructed in the MegAlign (DNA Star™ software) according to the CLUSTAL-algorithm (Higgins and Sharp 1988). This algorithm is based on the unweighted pair group method with arithmetic mean (UPGMA). The analysis was performed with residue weights of PAM250 and default penalties. Sequences for the comparisons were obtained from the EMBL/GenBank database under the accession numbers given in the legend of the corresponding Figure.

Results

Isolation of trout Igl cDNA clones

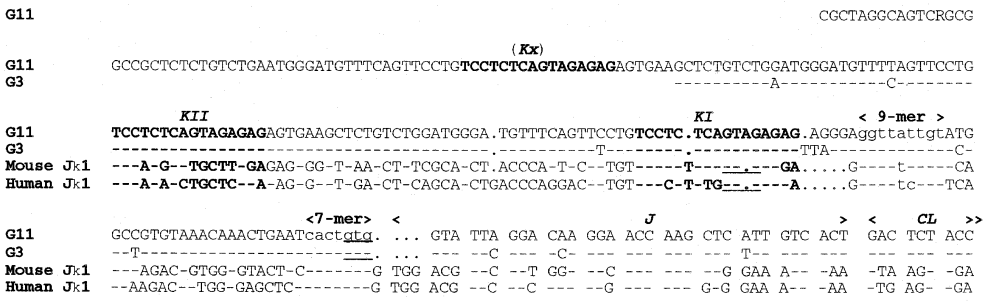
An amplified DNA fragment (about 600 bp) was obtained from trout thymocyte total RNA by reverse transcription-PCR. The amplified material was cloned and numerous clones were sequenced. Some of the clones contained the constant region of a known trout L chain isotype (Daggfeldt et al., 1993). Other clones (e. g., clone 2.6) encoded part of the *C*-like segment of a novel L chain with 255 nucleotides



of untranslated sequence which contained a polyadenylation signal and a polyA+ tail. The *Eco* RI–*Eco* RV fragment of clone 2.6 was used as a probe to screen a trout spleen cell cDNA library. Several positive clones were purified and sequenced, including a full-length clone, a 5′ truncated clone, two germline *J-C* transcripts, and a germline *C* transcript. The sequences of several complete *Igl V* regions were then obtained by RACE-PCR.

Fig. 2 Comparison of amino acid sequences of the trout *VL2a* and *VL2b* segments with VL sequences of different vertebrate species. Residues identical to the trout VL2a sequence are denoted by *hyphens*, and spaces introduced to optimize similarity between sequences are indicated by *dots*. Putative β strands, FR/CDR regions, and the numbers of the most conserved residues (Kabat et al. 1991) are indicated *above* sequences. The stretch of 11 amino acid residues corresponding to positions 71A to 71K in the VL2a sequence is *underlined*. *Under* sequences, residues conserved in most sequences are indicated by =; residues involved in intra-domain interactions are indicated by *; and residues involved in inter-domain interactions are indicated by + (Satow et al. 1986). GenBank accession numbers are U69987 (trout *VL2a*); U69988 (trout *VL2b*); X65260 (trout *VL1*); L25533 (catfish *VL*); M94393 (*Xenopus* Vρ). The *Xenopus* Vσ2 sequence is from Schwager and co-workers (1991). The mammamlian sequences are from Kabat and co-workers (1991); with page and entry (#) numbers as follows: mouse λ1: p. 264, # 3; human λ4: p. 144, # 1; rabbit λ2: p. 290, # 3; mouse κ4: p. 201, # 1; human κ1: p. 103, # 7; rabbit κ2: p. 277, # 24

Fig. 3 Alignments of trout germline *JL* sequences (G3 and G11) with the mouse and human *Jk1* germline sequences (Weaver and Baltimore 1987). The conserved 16 pb *K* sequences are indicated in *bold*, and the putative in-phase alternative translation initiation codons (GUG) are *underlined*. Residues identical to the clone G11 sequence are denoted by *hyphens*, spaces introduced to optimize alignments are indicated by *dots*



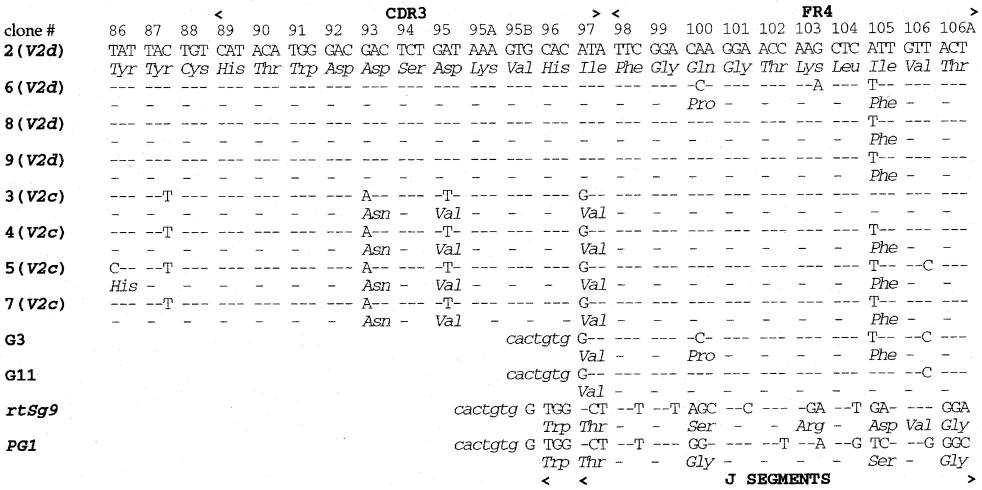


Fig. 4 Nucleotidic sequence alignment of *VL2d-JL* (clones 2, 6, 8, and 9) and *VL2c-JL* (clones 3, 4, 5, and 7) sequences. The sequences are compared with the trout germline *G3* and *G11* sequences (Figure 3, this paper) and with the trout *rtSgE* (Daggfeldt et al. 1993) and catfish *PG1* (Ghaffari and Lobb 1993) germline sequences. See text for explanations

Analysis of the IgLV regions

The sequences of ten clones encoding the complete *IgLV* regions were assigned to four different VL subgroups (VL2a–VL2d; Fig. 1). They share 83%–95% residues and thus form one single VL family, designated VL2. Sequence differences between the VL2a–d chains were mainly located in the putative CDR regions, as defined by Kabat's numbering system (Kabat et al. 1991). The FR regions of the four sequences were almost identical. These new VL2 domains (Fig. 2) had only 33.3% identical amino acid identity with the previously described trout VL1 domain (Daggfeldt et al. 1993). The FR3 regions had a stretch of 11 amino acid residues (positions 71A to 71K in Figure 2) with no obvious similarity to any other described vertebrate L chain. This peptide lay between the putative D and E β strands and contained mostly charged or hydrophilic residues. Most of the VL chain amino acid positions considered to be important for intra- and interdomain interactions in mammals (Satow et al. 1986) were conserved in trout *VL2* segments (Fig. 2).

Identification of J-C and C transcripts

Two putative germline transcripts were identified in clones G3 and G11 from the cDNA library; they contained sequences 5′ to the *JL* segments spliced to *CL2* segments (Fig. 3). Conserved heptamer and nonamer sequences separated by 23 bp were found 5′ to the *JL* sequences.

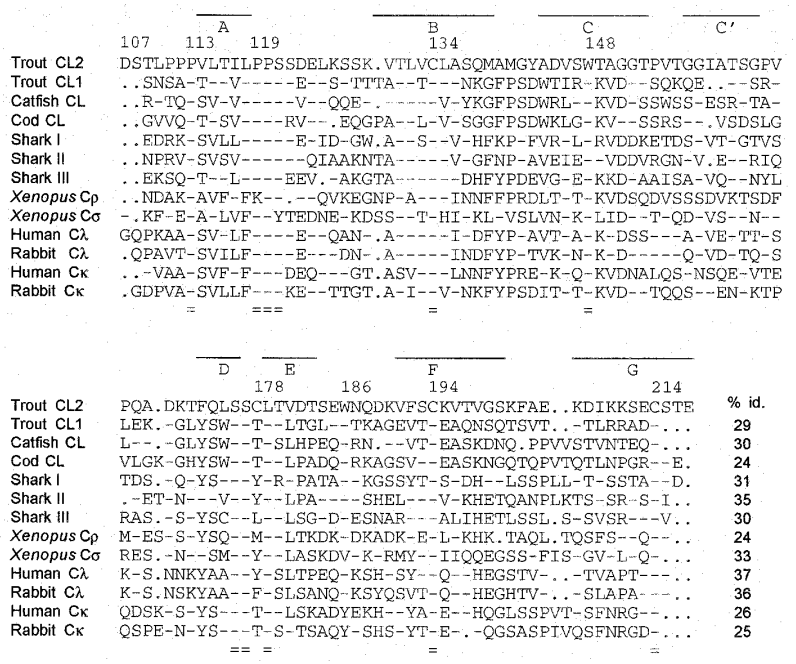


Fig. 5 Multiple alignment of the amino acid sequences of the trout CL2 region with CL sequences of different vertebrate species. Residues identical to the trout CL2 sequence are denoted by *hyphens*, and spaces introduced to optimize similarity between sequences are indicated by *dots*. Important residues are numbered above sequences (Kabat et al. 1991), and the conserved residues are indicated by = under sequences. GenBank accession numbers are: U69987 (trout CL2); X65260 (trout CL1); X68513 (cod CL); L25533 (catfish CL); M64307, L25557, L25561 (horned shark I, II and III, respectively); M94392 (*Xenopus* Cρ). The *Xenopus* Cσ sequence is from Schwager and co-workers (1991). Mammalian sequences are from Kabat and co-workers (1991), with page and entry (#) numbers as follows: human Cλ: p. 654, # 24; rabbit Cλ: p. 655, #54: human Cκ: p. 647, #1; rabbit Cκ: p. 648, #38

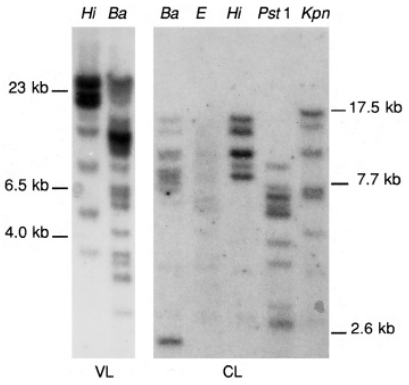


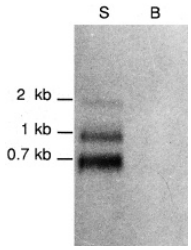
Fig. 6 Southern blot of trout erythrocyte DNA, digested with *Hin*dIII (Hi), *Bam* HI (Ba), *Eco* RI (E), *Pst* 1 or *Kpn*, and hybridized to ^{32}P-labeled probes corresponding to the variable (VL), or constant (CL) domains of the trout L2 chain

Upstream of the nonamers, the two clones had near identical 53 bp repeats, including a three-time (clone G11) or twice (clone G3) repeated 16 bp sequence (*KI*, *KII*, and *Kx*) very like the *KI* and *KII* nucleotide sequences found at a similar location upstream of the mouse and human *Jk1* germinal sequences (Weaver and Baltimore 1987). Clone G10 contained a complete *CL2* sequence followed by a complete 3′ untranslated sequence, with a polyadenylation signal and a Poly A^+ stretch. This *CL2* transcript was preceded by 131 nucleotides and contained an AG splicing signal at the 3′ end of the *J CL2* intron (data not shown).

J segments and VJ junctions

VL2 is associated with novel *JL* segments which differ from the published trout JL sequences. Figure 4 shows the nucleotide and amino acid sequence alignments of eight *VL2-JL* junctions from the clones obtained by RACE-PCR. These clones represent independent *VL2-JL* rearrangement events, since their sequences markedly diverged at the *VL-JL* junction (data not shown). The eight clones were divided into two subgroups, i. e., *VL2c* (clones 3, 4, 5, and 7), and *VL2d* (clones 2, 6, 8, and 9). Variants of the *JL* sequences are randomly associated with both the *VL2c* or *VL2d* sequences. The associated *JL* segments are one codon shorter than published *JL* segments of trout (Daggfeldt et al. 1993) and channel catfish (Ghaffari and Lobb 1993); the proteins have the typical sequence encoded by *J* segments, including the conserved Phe98, Gly99, Gly101, and Thr102 residues. Figure 4 shows that all the *VL2c* and *VL2d* clones have a histidine codon at position 96, which is most likely encoded by *VL*. The two groups of clones differ at position 97, which corresponds to the *VL2-JL* junction Codon 97 (GTA/Val) may be provided, at least in *VL2c-J L* junctions, by the first nucleotide triplet which follows in 3′ the heptamer recombination signal sequence of the *JL* segments (sequences G3 and G11 in Figure 3). The two 3′ residues of codon 97 (TA of ATA/Ile) in the *VL2d-JL* junctions could be provided by the *JL* segments, but the 5′ residue (A) could come from the *VL2d* segments.

Fig. 7 Northern blot analysis of trout L2 chain expression. Total RNA from trout spleen (lane S) and brain (lane B) was hybridized to a ^{32}P-labeled probe corresponding to the complete (VL2 + CL2) L2 chain



Structure of the CL2 domain

Since the new trout CL markedly differ from trout CL described by Daggfeldt and co-workers (1993), we designated them CL2 and CL1, respectively. The two trout CL isotypes share only 29% identical residues (Fig. 5). CL2 matches best to horned shark type II CL chain (35%) and the mammalian Cλ chains (36%–37%). Figure 5 also shows that the characteristic CL residues, such as Pro113, Cys134, Trp148, Cys194, and Cys214, are conserved in the trout CL2 isotype. Other typical CL residues are also well conserved, like the LPPS (118–121) and TLVCLA (131–136) stretches, as well as Ser176, Ser177, and Leu179. The CL2 chain has 217 residues and a unique Glu terminal residue. Other conserved residues of vertebrate CL chains are not conserved in the trout CL2 isotype, like Pro141 (replaced by Met), or residues 149 and 151 which are replaced by small hydrophilic residues (Thr and Gly). Remarkably, there is an extra-cysteine residue at position 178.

Southern blotting experiments

DNA blots from an individual trout were hybridized with probes specific for the *VL2* and *CL2* regions (Fig. 6). About 10–13 DNA segments were labeled with the *VL2* probe, depending on the restriction enzyme, and about eight bands were labeled with the *CL2* probe. These results indicate that the trout *L2* gene structure is complex, presumably similar to the clustered organization of the *L1* locus.

Northern blotting experiments

In spleen RNA, a strong, intermediate, and faint hybridization signal of ~0.7 kilobase (kb), 1 kb, and 2 kb, respectively, were observed (Fig. 7). They might correspond to *JLCL* or *CL* transcripts, fully processed RNA, and pre-mRNA, respectively. Faint signals for 0.7 and 1 kb transcripts were also detected in thymocyte RNA (data not shown), but none in brain.

Phylogenetic analysis

The trout VL2a and CL2 nucleotide and amino acid sequences were compared with the entire GenBank and NBRF databank sequences using the FASTA comparison program (Pearson and Lipman 1988). The best match for

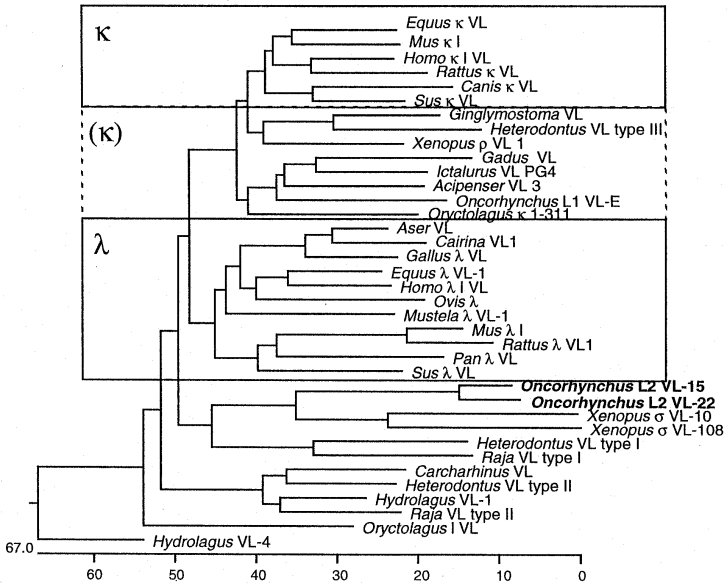


Fig. 8 Distance tree for amino acid sequences of the *VL* segments of different species. The trout L2 is shown in *bold print* and branches of the classical κ and λ are *boxed*. The branch of VL in other species closely related to κ is *hatched*. The sequences were obtained from the EMBL/GenBank databases under the following accession numbers: M59321, X75612, X05554, Z11895, M87784, L25533, X90556, X65260, K01359, L16765, L25561, X82069, M25720, M21075, L07563, M60441, X56270, X65288, M77360, M59322, X57729, M24368, L25568, M29049, L25560, L25556, L25566, and L25552. The sequence of *Canis* κ is from Wasserman and Capra (1978); *Xenopus* σ is from Schwager and co-workers (1991), and *Gadus* VL-16 is unpublished sequence from L. Pilström's lab. The x-axis denotes distance between the sequences in arbitrary units

the *VL2a* nucleotide sequence was a segment of the trout *VL1* sequence (Clone *rtSg 10*, *opt* = 144, 57.1% identity in 168 nucleotides overlap). Less significant scores (*opt* = 121-111) were found for variants of the *Xenopus laevis* Lσ (L2) variable segments (Schwager et al. 1991). The trout *CL2* nucleotide sequence had a significant score (*opt* = 199) with the sandbar shark type II *CL* segment (Rast et al. 1994), and with the cod (Daggfeldt et al. 1993) and the channel catfish (Ghaffari and Lobb 1993) CL chains. There were no significant FASTA scores between the trout *CL1* and *CL2* isotype nucleotides and between the trout *CL2* and the mammalian λ or κ isotypes. The best scores for the trout VL2a chain amino acid sequence was the *Xenopus* Vσ1 sequence (Schwager et al. 1991), followed by a majority of mammalian Vκ sequences. The trout CL2 amino acid had the highest degree of similarity to a large number of mammalian Cκ sequences (e. g., 38% identity for the human Cκ KERN (Ker^+/Oz^-) sequence in a 102 residues overlap, *opt* = 246).

Since it was difficult to classify unambiguously the trout VL2 and CL2 sequences using similarity comparisons, we built distance trees from vertebrate L chain amino acid sequences. This analysis confirmed the structural relationships between the trout VL2a and *Xenopus* Vσ (VL2) regions (Fig. 8). These, like other VL sequences of shark, cannot be readily linked to the canonical mammalian κ or λ isotypes. The trout VL1 sequence was more related to κ. The tree corroborated that trout CL2 is best related to *Xenopus* Cσ (Fig. 9). In contrast, trout CL1 sequence, like other teleost CL sequences, seemed to be more related to κ sequences.

Discussion

The initial PCR experiments were performed to see whether *TCRB*-like sequences could be amplified from rainbow trout thymocyte cDNA using a strategy that worked for the *TCRB* genes in the Mexican axolotl (Fellah et al. 1993). Incidentally, some of the clones from amplified thymus cDNA had an open reading frame encoding a putative IgL-like constant segment that was structurally different from the known trout CL isotype, called CL1 in this paper (Daggfeldt et al. 1993). The fact that these sequences were isolated from thymus cDNA is not surprising, since subsequent northern hybridization confirmed the presence of *L2* transcripts in trout thymus RNA. Complete *L2* sequences were obtained by anchored PCR and by screening a trout spleen cell cDNA library. Four *VL2* segments were detected; they belonged to the same family, but differed in their CDR1 and CDR2 putative regions, suggesting that functional selective pressure had favored their diversification.

A particular feature of the trout *VL2* segments is an unusual stretch of 11 amino acids in their FR3 region (positions 71A–71K in Figure 2). This stretch is predicted to form a coiled structure lying between the presumptive D and E β strands. This loop is rich in charged (Arg, Lys, Asp) and hydrophilic (The, Ser, Ala) residues and could be near the CDR1–3 loops that form the antigen recognition site of the V domain. The four VL2 sequences (Fig. 1) have different CDR1–2 loops, but identical 71A–71K regions. This additional loop of active residues is probably not

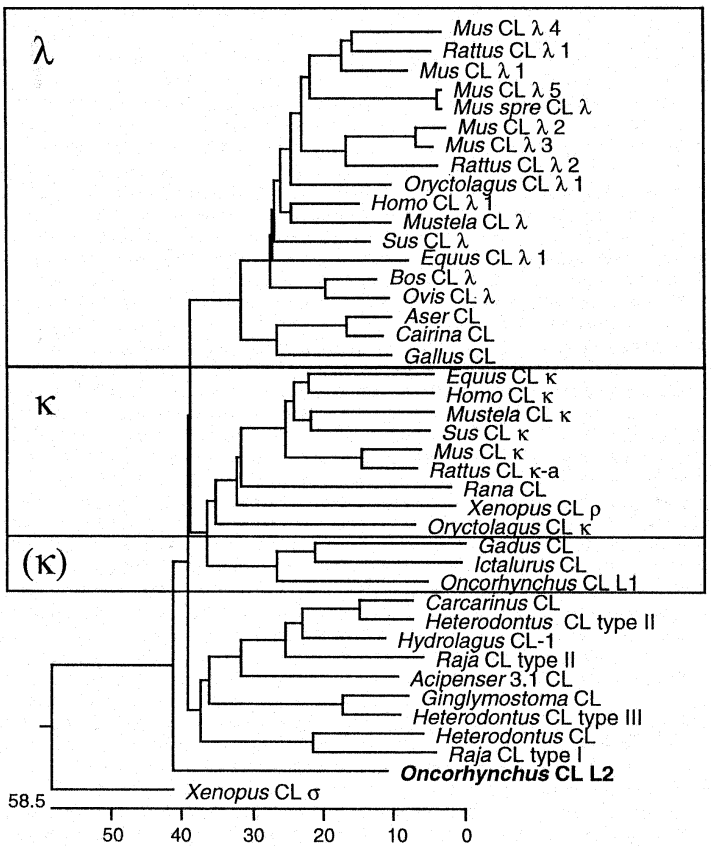


Fig. 9 Distance tree for amino acid sequence of the CL domain of different species. The trout L2 is shown in *bold print* and branches of the classical κ and λ are *boxed*. The branch of CL domains of teleost fish closely related to κ is *hatched*. The sequences have been obtained from the EMBL/GenBank databases under the following accession numbers: X58416, M22520, X58411, M30387, X58414, M16554, X58415, M22521, M12765, X51755, L07790, M59322, L07563, X62917, X82069, M25726, M33049, M81314, L25559, L25550, L25566, X90556, L16765, L25561, X15315, L25568, X75612, Z11903, L07787, M59321, X67003, J02574, M94392, X68511, L25533, and X65260. The sequence from *Ovis* λ is from Foley and Beh (1989) and *Xenopus* σ from Schwager and co-workers (1991). The x-axis denotes distance between the sequences in arbitrary units

directly involved in antigen recognition, but could be involved in an interaction with some invariant structure.

The presence of conserved *K* sequences 5′ to the non-rearranged *JC* transcripts in the trout is intriguing. The three trout 16 bp *KI*, *KII*, and *Kx* sequences are identical and separated by 37 bp identical sequences (Fig. 3). They are similar to the human and mouse *KI* and *KII* sequences that flank in 5′ the nonamer recombination signal of the *Jk1* gene, and which are also separated by a non-conserved 37 bp sequence (Leclercq et al. 1989). Guanine methylation interference assays have demonstrated that the human *KI* and *KII* sites have potential guanine residue contacts on both strands of the DNA and form a 16 bp protein binding site with an imperfect inverted symmetry (Weaver and Baltimore 1989). This structure, which is well conserved in the trout (*KI*: 5′-TCCTCTCAGTAGAGAG), is thought to act as a binding site for KLP, a B-cell-specific nuclear protein (Weaver and Baltimore 1989). The structure and function of the KLP protein are unknown in mammals but the conservation of its putative DNA binding site in a teleost fish suggests that it could be important for expression of the associated light chain genes. Although the trout *L2* gene is not closely related to the mammalian *Lk* genes, the teleost fish *L2* and the mammalian *Lk* genes could come from a common ancestral *IgL* locus. No *K*-like sequence was found 5′ to the recently described trout *JCL1* (Daggfeldt et al. 1993) and catfish (Ghaffari and Lobb 1993) germline *JCL* transcripts.

It has recently been demonstrated that human B-cell germline *JCk* transcripts encode a surrogate 15 000 M_r κ protein which can be expressed on the surface of pre-B cells in combination with μ heavy chains (Francès et al. 1994). Translation of the κ protein starts from a non-AUG alternative codon (GUG) located in the *KI* sequence, in frame with *Jk1*. A putative non-canonic GUG translation initiation codon is present in the trout heptamer sequence immediately 5′ to the first *J* codon (Fig. 3). This suggests the existence of a surrogate JLC2 protein in the rainbow trout.

Interpretation of the *VL-JL* junctions (Fig. 4) is based on preliminary data about the structure of the *VL2* and *JL2* genes (data not shown). Differences between the two groups of junctions (*VL2d* and *VL2c* sequences) at position 97 probably reflect differences at the 3′ ends of the *VL2d* and *VL2c* gene segments. Similarly, the different *JL* identified in this study might originate from multiple *JL* segments or reflect allelic polymorphism. Yet the *VL-J* junctions have limited diversity, with no clear nucleotide trimming and/or *N*-nucleotide addition (Fig. 4). On the other hand, *N*-nucleotide addition is a common feature of the *VDJ* junctions in rainbow trout IgH μ and Tcr β chains (Roman et al. 1995; Partula et al. 1995).

The amino acid sequence comparison confirms that the trout L2 chains cannot be classified as mammalian κ or λ chain isotypes. Elasmobranchs, the most primitive vertebrates in which *IgL* genes have been extensively analyzed, have three L chain types (Rast et al. 1994). Type I L chains appear to be an ancestral sister group of all other L chains; type II chains seem to be more related (although still distant) to mammalian and avian λ chains, and type III chains match the mammalian κ chains. Recent distance

trees of vertebrate L chains (Daggfeldt et al. 1993; Rast et al. 1994), and the distance trees in this paper, place the roots of the channel catfish and the cod L chains as well as the trout L1 chains near the mammalian κ chains, indicating that they are structurally related to each other and to elasmobranch type III and *Xenopus* ρ chains. The trout L2 chains, although sequence-related to L1 chains, are most closely related to elasmobranch type I and *Xenopus* σ chains. The present results provide an additional element in the phylogenic classification of trout L2 chains: i. e., the striking conservation of putative regulatory *K* structures between the genomic sequences located 5′ to the trout *JL2* and mammalian *Jk1* genes. Hence, these regulatory *K* structures probably arose from *K*-like ancestor genes in the primitive common ancestors of bony fish and mammals.

The Southern experiments suggest that the trout *L2* genes, similar to *L1* genes, and the channel catfish and cod *L* genes have a cluster-like genomic organization, with multiple *V*- and *C*-related genes (Ghaffari and Lobb 1993; Daggfeldt et al. 1993). Thus, although bony fish probably have a mammalian-like single-locus *IgH* gene organization with a limited number of *CH* segments, their *IgL* genes have a more complex organization reminiscent of the clustered organization of the cartilaginous fish *IgL* genes (Rast et al 1994). However, it is still unknown whether bony fish *IgL* genes are organized in a single-locus containing multiple interspersed *V, J*, and *C* genes, as in the mammalian *Ll* genes, or separated into several independent loci, as in sharks. Preliminary studies of genomic clones indicate that trout *L2* has two *VL* segments 5′ of each *CL* segment, as in the channel catfish. However, the direction of the transcription seems to be the same for the two *VL* segments, unlike that in the channel catfish (Ghaffari and Lobb 1993).

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References

Andersson, E., and Matsunaga, T. Complete cDNA sequence of a rainbow trout *IgM* gene and evolution of vertebrate IgM constant domains. *Immunogenetics 38:* 243–250, 1993

Daggfeldt, A., Bengten, E., and Pilström, L. A cluster type organization of the loci of the immunoglobulin light chain in Atlantic cod (*Gadus morhua* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum) indicated by nucleotide sequences of cDNAs and hybridization analysis. *Immunogenetics 38:* 199–209, 1993

Fellah, J. S., Kerfourn, F., Guillet, F., and Charlemagne, J. Conserved structure of amphibian T-cell antigen receptor β chain. *Proc Natl Acad Sci USA 90:* 6811–6814, 1993

Foley, R. C. and Beh, K. J. Isolation and sequence of sheep IgH and L chain cDNA. *J Immunol 142:* 708–711, 1989

Francés, V., Paudran-Garcia, D., Guret, C., Ho, S., Wang, Z., Duvert, V., Sacland, S., and Martinez-Valdez, H. A surrogate 15 kDa JCκ protein is expressed in combination with μ heavy chain by human B cell precursors. *EMBO J 13:* 5937–5943, 1994

Frohman, M. A., Dush, M. K., and Martin, G. R. Rapid production of full-length cDNAs from rare transcripts: amplifications using a single gene-specific primer. *Proc Natl Acad Sci USA 28:* 8998–9002, 1988

Ghaffari, S. H. and Lobb, C. J. Structure and genomic organization of immunoglobulin light chain in the channel catfish. An unusual genomic organizational pattern of segmental genes. *J Immunol 151:* 6900–6912, 1993

Havarstein, L. S., Aasjord, P. M., Ness, S., and Endressen, C. Purification and partial characterization of an IgM-like serum immunoglobulin from Atlantic salmon (*Salmo salar*). *Dev Comp Immunol 12:* 773–785, 1988

Higgins, D. G. and Sharp, P. M. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene 73:* 237–244, 1988

Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S., and Foeller, C. *Sequences of Proteins of Immunological Interest (5th edn)*, USDHHS, Public Health Service, National Institute of Health, Bethesda 1991

Leclercq, L., Butkeraitis, P., and Reth, M. A novel germ-line JK transcript starting immediately upstream of Jκ1. *Nucleic Acids Res 17:* 6809–6819, 1989

Lobb, C. J., Olson, O. J., and Clem, L. W. Immunoglobulin light chain classes in a teleost fish. *J Immunol 132:* 1917–1923, 1984

Partula, S., De Guerra, A., Fellah, J. S., and Charlemagne, J. Structure and diversity of the T cell antigen receptor β-chain in a teleost fish. *J Immunol 155:* 699–706, 1995

Pearson, W. R. and Lipman, D. J. Improved tools for biological sequence comparison. *Proc Natl Acad Sci USA 85:* 2444–2449, 1988

Rast, J. I., Anderson, M. K., Ota, T., Litman, R. T., Margittai, M., Shamblott, M. J., and Litman, L. W. Immunoglobulin light chain class multiplicity and alternative organizational forms in early vertebrate phylogeny. *Immunogenetics 40:* 83–99, 1994

Roman, T., De Guerra, A., and Charlemagne, J. Evolution of specific antigen recognition: size reduction and restricted length distribution of the CDRH3 regions in the rainbow trout. *Eur J Immunol 25:* 269–273, 1995

Sànchez, C., Dominguez, J., and Coll, J. Immunoglobulin heterogeneity in the rainbow trout, *Salmo garidneri* Richardson. *J Fish Dis 12:* 459–465, 1989

Sànchez, C. and Dominguez, J. Trout immunoglobulin populations differing in light chains revealed by monoclonal antibodies. *Mol Immunol 28:* 1271–1277, 1991

Sanger, F., Nicklen, S., and Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA 74:* 5464–5467, 1977

Satow, Y., Cohen, G. H., Padlan, E. A., and Davies, D. R. Phosphorylcholine binding immunoglobulin Fab McPC603. *J Mol Biol 190:* 593–604, 1986

Schwager, J., Bürckert, N., Schwager, M., and Wilson, M. Evolution of immunoglobulin light chain genes: analysis of *Xenopus* IgL isotypes and their contribution to antibody diversity. *EMBO J 10:* 505–511, 1991

Shamblott, M. J. and Litman, G. W. Genomic organization and sequences of immunoglobulin light chain genes in a primitive vertebrate suggest coevolution of immunoglobulin gene organization. *EMBO J 8:* 3733–3739, 1989

Wasserman, R. L. and Capra, J. D. The amino acid sequence of the light chain variable region of a canine myeloma immunoglobulin: evidence that the Vκ subgroups predated mammalian speciation. *Immunochemistry 15:* 303–305, 1978

Weaver, D. and Baltimore, D. B lymphocyte-specific protein binding near an immunoglobulin κ-chain gene J segment. *Proc Natl Acad Sci USA 84:* 1511–1520, 1987