Immunogenetics 38: 199–209, 1993

Immuno-genetics

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

Annika Daggfeldt[1], Eva Bengtén[1], Lars Pilström[1, 2]

[1] Department of Immunology, BMC, Uppsala University, Box 582, S-751 23 Uppsala
[2] Klubban Biological Station, Östersidan, PL 2535, S-450 34 Fiskebäckskil

Received November 26, 1992/Revised version received February 4, 1993

Abstract. Antibody screening and colony hybridization of cDNA libraries have been used to isolate clones of the immunoglobulin light (IgL) chain from Atlantic cod (*Gadus morhua* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum). Sequence analysis shows dissimilarities in the constant part of the molecule (CL) within each species. Comparisons of the amino acid sequences of the constant parts of the IgL chains show a 55% identity between the two teleost species. When compared with other species the highest similarities are found to the constant domain of the IgL chain from mammals (30%–37%), but the teleost IgL chain can be classified neither as κ nor λ. The VL domain in Atlantic cod and rainbow trout is also more similar to those of mammals than to those of other animal species, but no difference between κ and λ was noticed. Genomic Southern blots hybridized with fragments coding for the constant part of IgL gave several bands larger than 2 kilobases and a similar pattern was obtained with fragments coding for the variable part. These results show that the locus of the IgL chain has a multiple organization in teleost fish and that the locus has an organization similar to that of sharks. Several of the cDNA clones isolated from both the head kidney and the spleen represent nonrearranged or nonspliced mRNA, and northern blot analysis shows that such transcripts are present in both the head kidney and the spleen.

Correspondence to: L. Pilström.

Introduction

The genomic organization of the immunoglobulin (*Ig*) heavy chain locus in vertebrates (except reptiles) can be divided into three main types, i.e., the elasmobranch type (Kokubu et al. 1988; Harding et al. 1990), the avian type (Reynaud et al. 1989), and the mammalian type (Earley et al. 1980; Kawakami et al. 1980). In the first type each of a variable (*V*), diversity (*D*), joining (*J*), and constant (*C*) segment form clusters of 10 kilobases (kb), which are repeated in the genome. In the second, avian type, there is only one functional *V, D, J,* and *C* segment and antibody diversity is generated by conversion of gene segments from a pool of pseudo-*V* genes upstream of the functional gene. In the mammalian type, clusters of *V* segments precede clusters of *D* and *J* segments followed by the *C* segments. The heavy chain locus of amphibia (Schwager et al. 1988) and teleost fish (Amemiya and Litman 1990; Wilson et al. 1990; Bengtén et al. 1991) is of the mammalian type, and the organization in reptiles is unknown.

Studies of the IgL chain gene have shown that the organization of this locus is similar to that found in the heavy chain locus in the different groups, i.e., the *V, J,* and *C* segments of elasmobranchs (*Heterodontus*) form clusters of 2.7 kb (Shamblott and Litman, 1989a, b), there is only one functional *V, J,* and *C* segment in birds (Reynaud et al. 1987), and in mammals several *V* segments precede some *J* segments with one *C* segment (Reviewed by Zachau et al. 1989; Selsing et al. 1989). In amphibians the organization of the L chain locus is of the mammalian type (Schwager et al. 1991), but no data are available about reptiles and about teleost fish.

Two isotypes of light chains (κ and λ) are known in mammals and they are encoded by different loci (Zachau et al. 1989; Selsing et al. 1989). The above classification of the organization of the IgL chain gene is a simplification, because the λ locus of mammals seems

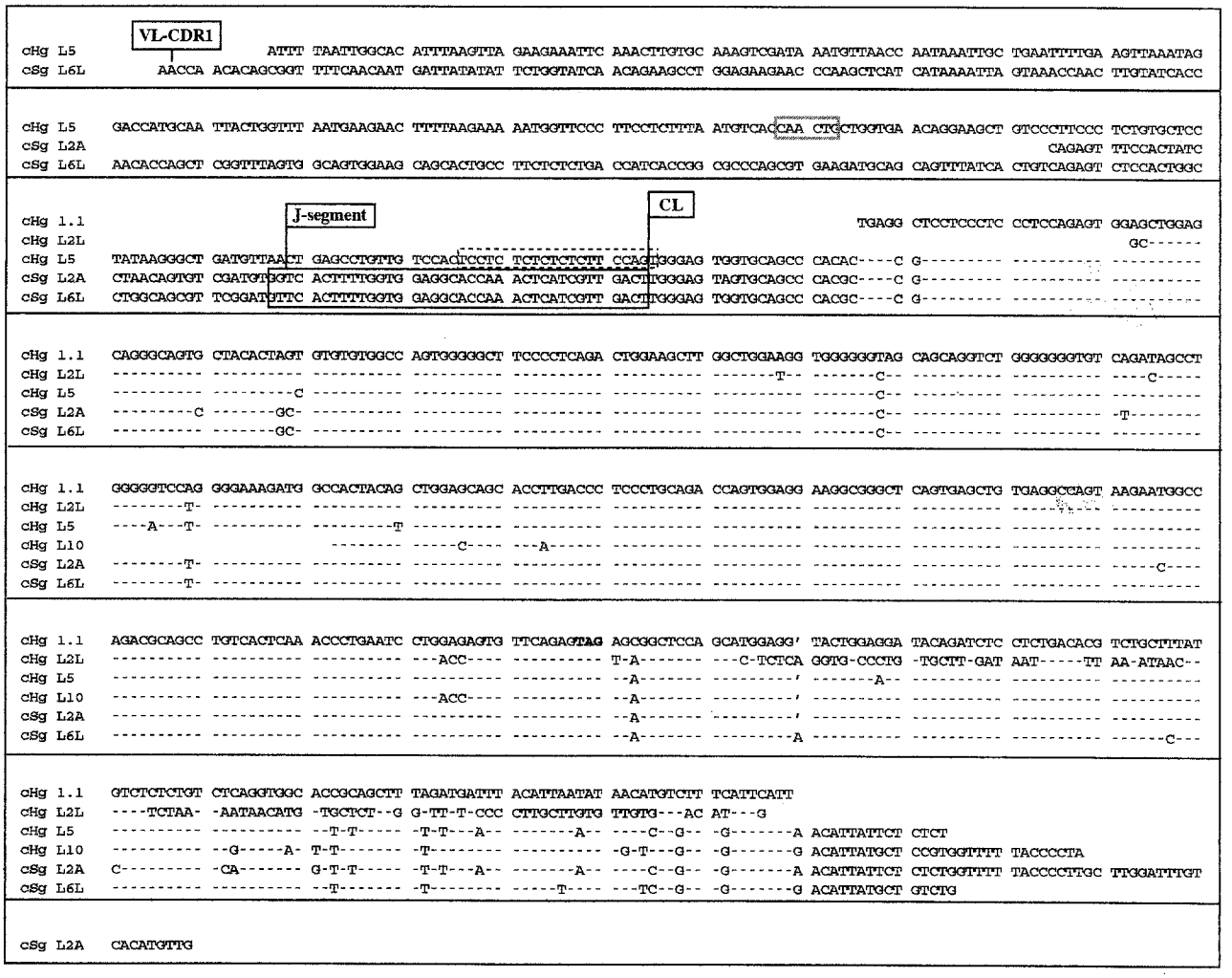


Fig. 1. Nucleotide sequence alignment of the IgL chain clones from cDNA libraries of head kidney and spleen from Atlantic cod (*Gadus morhua* L.). The sequences are aligned based on the stop codon (*bold print*) of the constant part of IgL. *Dashes* indicate nucleotide identity to clone cHg 1.1, and *apostrophe signs* denote gaps introduced to optimize the alignment. The V_L of clone cSg L6L and the putative start of the constant domain of the IgL chain is indicated by C_L. The *J* segment is *boxed* and the *hatched box* indicates the splice signal sequence. The *grey box* (second section from top) shows the E-box core motif. These nucleotide sequences are available from the EMBL, GenBank, and DDBJ nucleotide sequence databases under the accession numbers X68511–X68516, respectively.

to be more complicated and contains from a few to several functional *C* segments and some pseudogenes (Selsing et al. 1989; Hayzer 1990). Although the λ locus is organized in a repetitive way (in mouse *V-J-C-J-C-V-J-C*), the gene duplication seems to have occurred at a later stage in evolution and is not related to the type found in *Heterodontus*, because different mammalian species have different organization of the λ locus (Hayzer 1990). Two isotypes of the light chain (ρ and σ) have also been described in the amphibian *Xenopus* (Schwager et al. 1991; Zezza et al. 1991). The organization of the loci for σ is of the mammalian type (Schwager et al. 1991) but that of ρ is so far unknown. However, σ is expressed in two closely related forms, each with its set of *V* genes, indicating a comparatively recent gene duplication (Schwager et al. 1991). By using monoclonal antibodies the light chain of channel catfish (teleost) has also been divided into two isotypes designated, for the time being, F and G (Lobb et al. 1984). Two putative isotypes of the light chain have also been described in the reptilian alligator (Saluk et al. 1970). The light chain from the elasmobranchs *Heterodontus* and *Carcharhinus* are most similar to λ of mammals (Schamblott and Litman 1989a; Hohman et al. 1992).

In this paper we present the first results regarding the IgL chain loci in two species of teleost fish. Sequencing of cDNA clones and Southern blot hybridization with these clones show that the locus has a complicated organization, with several repeats of the

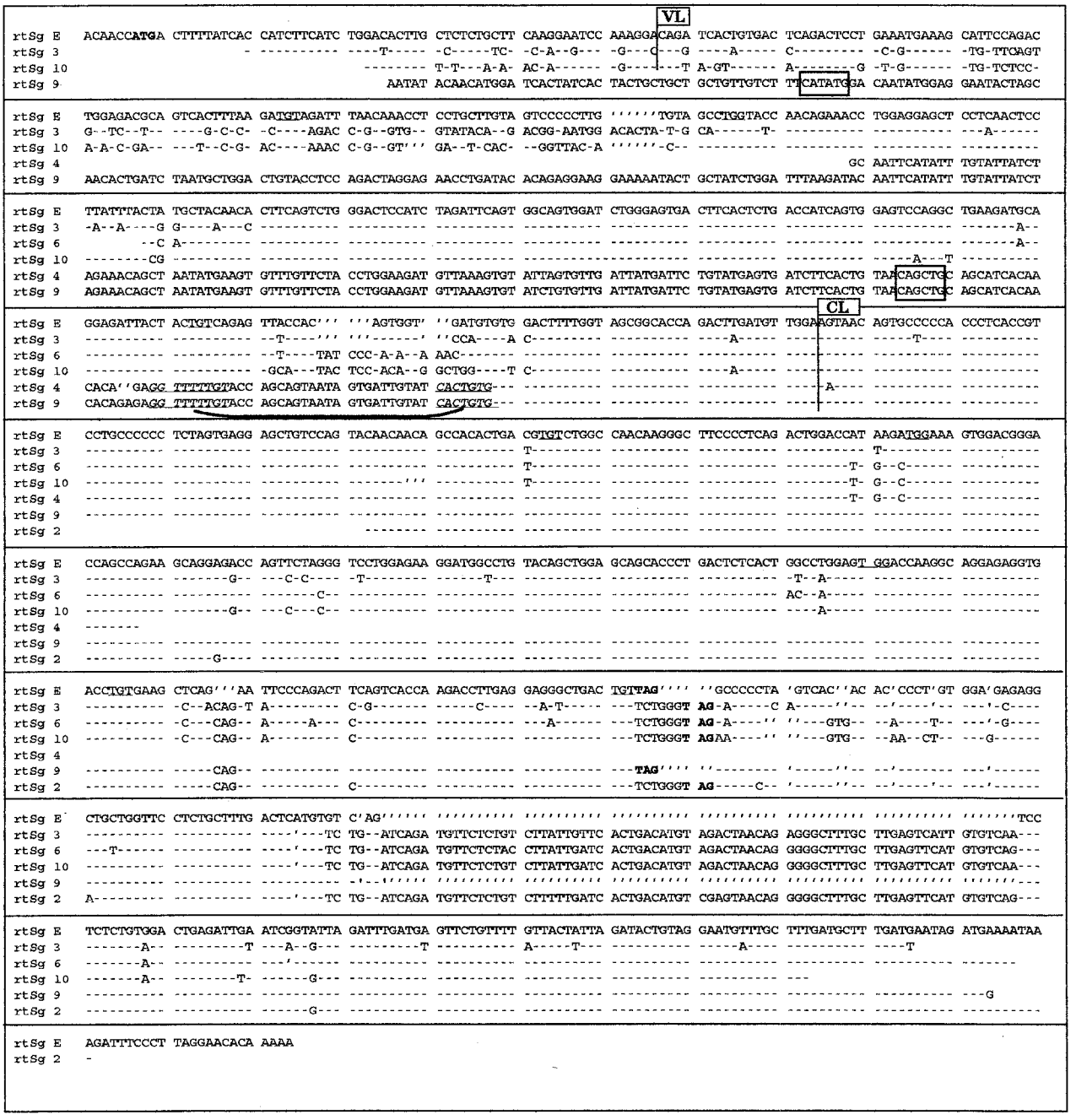


Fig. 2. Nucleotide sequence alignment of the IgL chain clones from cDNA libraries of head kidney and spleen from rainbow trout (*Oncorhynchus mykiss*). The sequences are aligned based on the conserved cysteine and tryptophan codons (*underlined*) of the constant part of the IgL. *Dashes* indicate nucleotide identity to clone rtSg E and *apostrophes* denote gaps introduced to optimize the alignment. The start and stop codons are in *bold print.* The hepatmer-nonamer recombination signal is indicated by *underlined italics.* Boxes show E-box core motifs. These nucleotide sequences are available from the EMBL, GenBank, and DDBJ nucleotide sequence databases under the accession numbers X65260 and X68517–X68522, respectively.

variable and constant domains indicating an organization similar to that found in sharks. The cDNA sequences show the presence of unrearranged transcripts in the head kidney and the spleen, and northern blot analysis also shows transcripts of three different sizes of the IgL chain.

Materials and methods

Animals and collection of tissue. Atlantic cod (*Gadus morhua* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum) were caught, kept, and killed as described by Bengtén and co-workers (1991) and Lee and co-workers (1993). In both cases the spleen, the head kidney

(pronephros), the liver, and for cod either the ovarium or the testis, were removed, frozen in liquid nitrogen, and stored at –70° C.

Antibodies. Antiserum against cod serum immunoglobulin (CS-Ig) was prepared in rabbits and the antibodies purified by affinity chromatography with CS-Ig coupled to Sepharose 4B (Pilström and Petersson 1991; Israelsson et al. 1991). Antiserum specific for the light chain was obtained by immunizing rabbits with the L-chain band from a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel of CS-Ig run under reduced conditions (Harlow and Lane 1988). The specificities of the isolated antibodies were determined by immunoblot analysis.

RNA and cDNA library preparation and analyses. Poly(A)$^+$ RNA and cDNA libraries were made from cod spleen and head kidney (Bengtén et al. 1991) as well as rainbow trout spleen (Lee et al. 1993) and screened in *Escherichia coli* Y1090 for expression according to Huynh and co-workers (1985) with rabbit anti-CS-Ig antibodies and a highly cross-reactive rabbit antiserum against Ig from brown trout (*Salmo trutta* L.; Thuvander et al. 1987), respectively. Commercial goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma, St. Louis, MO) was used as secondary antibody. One of the clones from cod (cHg 1.1) isolated by this technique was used as probe for rescreening of the cod libraries together with a rabbit antiserum specific for the light chain of cod. The cDNA clones obtained from rainbow trout with the antibody screening were rescreened by hybridization with a cDNA fragment encoding the main part of the secretory IgH from Atlantic salmon (*Salmo salar* L.). One clone (rtSg E), which showed a strong signal with the antiserum but no signal with the probe from salmon indicating a putative L-chain clone, was used as the probe for rescreening of the rainbow trout library. Positive clones were isolated, and those selected for further analysis were subcloned into pGemini blue (Promega, Madison, WI).

Probes. For the Southern and northern blots *V*- and *C*-specific probes were used. These were for cod cDNA fragments containing nucleotides 1–256 of clone cSg L6L and nucleotides 1–325 of clone cHg 1.1, respectively (Fig. 1) and for rainbow trout, nucleotides 1–254 and nucleotides 457–587 of clone rtSg E, respectively (Fig. 2). The different probes were labeled by random priming according to Feinberg and Vogelstein (1983).

Southern blot. Genomic DNA from cod erythrocytes and rainbow trout liver was isolated, as described by Sambrook and co-workers (1989) and digested with restriction enzymes. The DNA was separated on an agarose gel and transferred to a nylon membrane Hybond-N+ (Amersham, Amserham, UK) by vacuum blotting with 0.4 M NaOH. The filters were prehybridized and hybridized in a medium (modified from Church and Gilbert 1984) containing 0.5 M $NaH_2PO_4/NaHPO_4$ (pH 7.2), 1 mM ethylenediaminetetraacetate (EDTA) and 7% (w/v) SDS with random primed cDNA fragments. The filters were washed in 1 × standard sodium citrate (SSC), 0.1% SDS at 65° C and autoradiographed.

Northern blot. Poly(A)$^+$ RNA from spleen, head kidney, liver, and ovary was fractionated on an agarose gel containing 8.6% formaldehyde, and transferred to a nylon membrane Hybond-N (Amersham) in 20 × SSC. The filters were fixed by UV cross-linking, prehybridized, hybridized, and washed as described for Southern blot. To ensure that similar amounts of poly(A)$^+$ RNA were present in each lane of the filters, an MEB 3 mouse ribosomal RNA probe was used as a control in the hybridization.

Sequencing. The Sanger dideoxy nucleotide chain termination method was used (Sanger et al. 1977) with T7 DNA polymerase, and the plasmid sequencing primers Sp6/T7. All the clones were sequenced in both directions and the nucleotide sequences were compared and translated into amino acid sequences with the MacMolly™ (Softgene, Berlin, Germany) and DNA Star™ software (DNA Star, London, UK).

Results

The IgL gene has duplicated several times in bony fish. cDNA libraries from the spleen and the head kidney of one Atlantic cod were screened with affinity purified antibodies against cod Ig. Several positive clones were found, most of which represented the Ig heavy chain (Bengtén et al. 1991). One of the clones (cHg 1.1) represented the main part of the constant domain of the light chain (Fig. 1). In order to get full-length clones of the light chain(s) of the putative two isotypes, we rescreened the libraries with cHg 1.1 as a probe and with a rabbit antiserum specific for the cod IgL chain (isolated by SDS-PAGE under reduced conditions). Several positive clones were isolated, and five of them, designated cHg L2L, cHg L5, cHg L10, cSg L2A, and cSg L6L, have been studied. The first three derive from a head kidney cDNA library and the two latter from the spleen library. The clones cHg L2L and cSg L6L were isolated by antibody screening with L-chain-specific antiserum, while clones cSg L2A, cHg L5, and cHg L10 were isolated by hybridization with cHg 1.1 as the probe. Sequence analysis of these clones revealed several irregularities in comparison to cHg 1.1. In the part coding for the constant domain (C_L) there are 9/280, 7/305, 5/152, 9/305, and 6/305 nucleotide dissimilarities, and in the 3', non-coding part, there are 10–86/129 nucleotide dissimilarities (Fig. 1). The dissimilarities of the coding region are not of such a magnitude that they indicate a presence of L-chain isotypes. Thus, these results indicate that the IgL chain locus of Atlantic cod is multiple similar to the λ locus in mammals. In order to get some idea of the degree of polymorphism, Southern blot analyses of genomic (erythrocyte) DNA were made with a probe representing the coding part of the C_L and with a probe representing the variable domain (V_L). The hybridized filters show that the locus is highly multiple, with at least 20 bands which are longer than 2 kb, some of which probably contain several copies, as judged from the intensity of the signal (Fig. 3). The pattern of the two filters are similar, particularly with fragments >2 kb (Fig. 3), which shows that the *IgL* locus has a clustered organisation of the V_L and C_L segments. Southern blot hybridization of genomic DNA from other individual cod gave similar but not identical banding patterns (data not shown).

To establish whether this type of organization is a general phenomenon in teleost fish or particular to Atlantic cod, a cDNA library from the spleen of one individual rainbow trout was screened with an an-

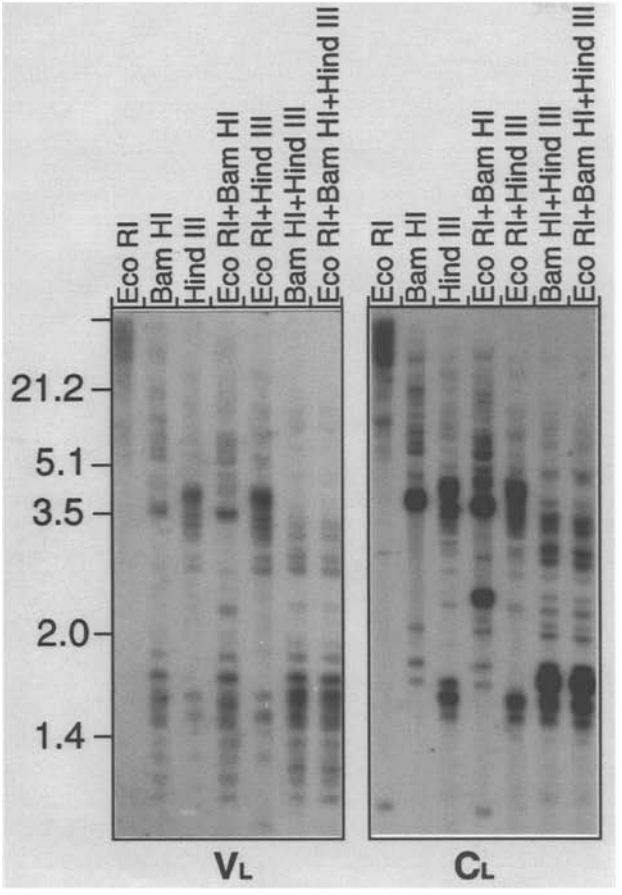


Fig. 3. Southern blot analyses of genomic (erythrocyte) DNA (10 µg per lane) of Atlantic cod, with probes containing the variable domain (*left*) and the constant domain (*right*) of IgL chain from cod. The restriction enzymes used are denoted above each lane and the position of size markers (in kb) are shown on the *left-hand* side.

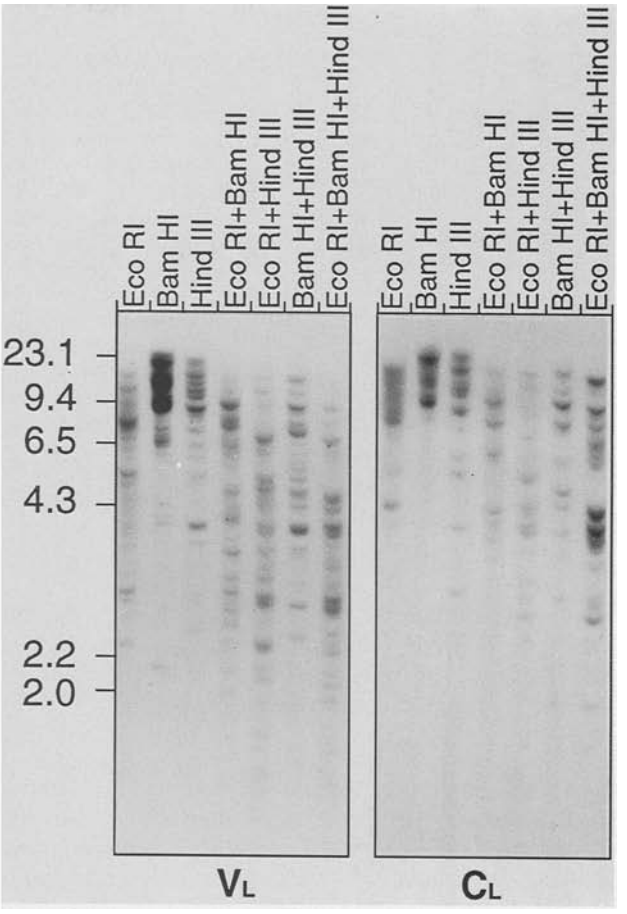


Fig. 4. Southern blot analysis of genomic (liver) DNA (10 µg per lane) of rainbow trout with a probe containing the variable domain (*left*) and the constant domain (*right*) of IgL chain from rainbow trout. The restriction enzymes used are denoted above each lane and the position of size markers (in kb) are shown on the *left-hand* side.

tiserum against Ig of brown trout, known to show cross-reactivity with Ig of rainbow trout (Thuvander et al. 1987, Lee et al. 1993). Several positive clones were isolated and those giving the strongest signal were re-screened with a 1300 bp cDNA probe coding for the Ig heavy chain from Atlantic salmon (Hordvik et al. 1992). One clone – rtSg E – was negative in this rescreening, indicating that it might represent the IgL chain of rainbow trout. Sequencing of rtSg E showed that it coded for the leader peptide, the whole VL, the CL, and the 3' non-coding polyA tail (Fig. 2). More clones were isolated from the same cDNA library with clone rtSg E as probe, some of which were selected for further analysis. As seen in Figure 2, there are IgL nucleotide dissimilarities in rainbow trout as is the case with Atlantic cod. This is particularly clear in the leader and first framework (FR1) of the VL and at the position of the stop codon. In the frameworks of the *VL*-region (excl CDRs) there are 30/246 nucleotide dissimilarities in the clones rtSg 3 and rtSg 10, respectively, in comparison with clone rtSg E. A comparison between the segments encoding the constant part of the L chain show 3–28 dissimilarities per 316 nt in the clones rtSg 3, 6, 10, 9, and 2 when compared with clone rtSg E. There is also a deletion of 73 nt in the non-coding 3' end of the clones rtSg E and rtSg 9 in comparison with the others, and, apart from this deletion there are 19/169, 20/182, 21/158, 5/179, and 8/186 nucleotide dissimilarities in this part of the clones (Fig. 2). Southern blot analysis was performed with genomic DNA from the liver of rainbow trout which was hybridized with the *VL* and *CL* fragments of rtSg E (Fig. 4). The hybridized filters show that the locus is repetitive with more than 10 bands longer than 2 kb as is the case in Atlantic cod (Fig. 3). The patterns of the VL and CL filters are very similar, showing that the organization of the *IgL* gene is clustered, more the elasmobranch type than the mammalian type, and not organized like the

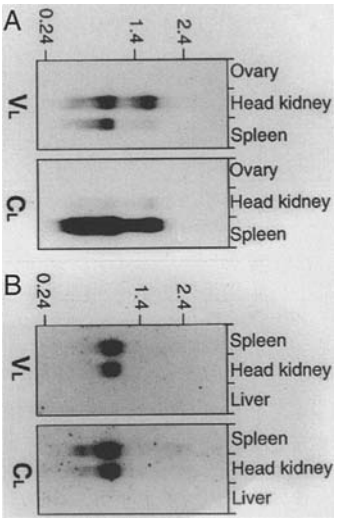


Fig. 5 A, B. Northern blot analysis from mRNA (2 μg/lane) of ovary, head kidney, and spleen from Atlantic cod (**A**) and of spleen, head kidney, and liver from rainbow trout (**B**) with probes encoding the variable (*left*) and constant (*right*) domain IgL chain. Size markers (in kb) are shown on the *left-hand* side. The MEB3 control showed approximately equal amounts of poly(A)$^+$-RNA in all lanes except in the head kidney sample of Atlantic cod hybridized with the *CL* probe, which was lower in comparison to the others.

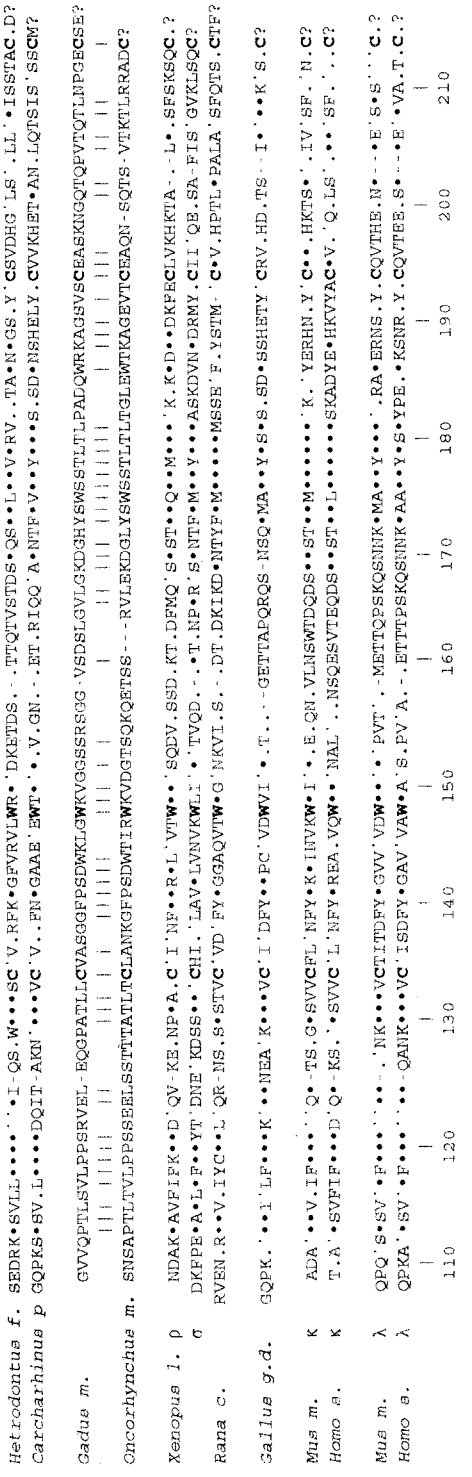
heavy chain of teleost fish. As in cod, there is a small variation between individual rainbow trout in the Southern blot hybridization (data not shown), indicating allelic variation in the population.

Some IgL genes are transcribed and spliced without rearrangement. The clone cSg L6L from cod and the clones rtSg E, rtSg 3, rtSg 6, and rtSg 10 from rainbow trout contain sequences which are homologous to mammalian VL upstream of the constant region. The clone cSg L2A from cod contain only 24 amino acids upstream the constant region but its sequence is very similar to FR4 and CDR3 of cSg L6L, indicating that it is rearranged and spliced as expected. The clone cHg L5 (from cod) has 250 nt 5' of the beginning of the constant domain i.e., it ought to code for most of the VL domain (Fig. 1). However, no such sequence could be found; instead, the splicing acceptor nucleotides TCCTCTCTCTCTCTTCCAGT were recognized (Sanapathy et al. 1990) at the putative start of the CL domain. Therefore, this clone may represent an unspliced mRNA. The clones rtSg 4 and rtSg 9 from rainbow trout contain the spliced *J* segment but the sequence upstream does not seem to code for any VL. Instead, the heptamer/nonamer recombination signal segment (RSS) is recognized in front of the *J* segment in these clones, showing that they represent *J-C*-spliced mRNA from unrearranged DNA.

Northern blot analysis was performed with mRNA from three different organs – spleen, head kidney and ovary (cod), or liver (rainbow trout) – in order to test whether the transcription and splicing of the nonrearranged IgL chain gene is common or simply something abnormal isolated by chance. As seen in Figure 5A, there is a main band at 1 kb of mRNA from the spleen of Atlantic cod but two equally intense bands at 1 kb and 2 kb in the head kidney when the filter is hybridized with a probe of *VL*. Hybridization with the *CL* probe gave bands similar to those with the *VL* probe in the head kidney, but gave a smear in the spleen with a slight banding at 0.5, 1, and 2 kb. In rainbow trout, the northern blot hybridization with a *VL* probe gave a strong band at 1 kb in both the spleen and head kidney (Fig. 5B). With the *CL* probe there are also fainter bands at 0.5 and 2 kb in the spleen and even fainter in the head kidney. The 1 kb band is the strongest in both species and most probably represents the correct transcripts for a functional (rearranged and spliced) L chain. The *CL* probes also hybridize to two additional bands at 0.5 and 2–2.5 kb, which are most obvious in Atlantic cod. The *VL* probes hybridize only to the longer transcript, indicating that it represents unrearranged/unspliced mRNA and that the short (0.5 kb) transcript only contains *CL* transcripts. The smeared appearance of the northern blot filters in Atlantic cod (Fig. 5A) demonstrates the presence of transcripts in the whole size range from 0.5 to >2.5 kb with *VL* and *CL* sequences.

Comparison of teleost IgL with IgL from other species. The constant domain. The deduced amino acid sequences show that the clone cSg L2A from Atlantic cod and the clone rtSg E from rainbow trout represent the constant domain of the IgL chain (Fig. 6; Table 1). The nucleotide exchanges seen in Figures 1 and 3 are seldom silent but often give rise to alterations of the amino acids as seen in Figures 7 and 8. In the Atlantic cod these differences seem to be more evenly distributed in the sequence than in rainbow trout, where the shifts occur at certain parts of the molecule. We have used the amino acid sequences of cSg L2A and rtSg E for the comparisons with the IgL of other species (Fig. 6; Table 1). The constant parts of the IgL chain from Atlantic cod and rainbow trout have 56 amino acids identical in their sequences, which correspond to a 55% similarity (Table 1). Cross hybridization at low stringency with the *CL* probe from Atlantic cod and genomic DNA from rainbow trout and vice versa gave very faint bands (data not shown), so there is no other light chain isotype with a higher degree of similarity in any of the two species. The CL domain of rainbow trout containing 102 amino acids is the shortest of those compared, which is mainly due to a deletion of three amino acids at position 160–163 (Fig. 6). Comparisons of the amino acid sequence of the two clones with those of the constant domain in the L chain of other species show similarities of 22%–37% with the highest values obtained for mammals (Table 1). The pattern of the distribution of hydrophobic and basic amino acids show that a normal folding of the CL domain is most probable in the Atlantic cod. In rainbow trout, however, three amino acids are missing in the area of the fourth β strand, and, in addition, the hydrophobic amino acid at the beginning of this strand is also missing.

Although the degrees of similarity are generally a little higher for the κ chain in mammals (mouse and humans), the L chain represented by cSg L2A and rtSg E can be classified neither as κ nor λ. In order to make a more detailed study of the relationship, the amino acid sequences of the constant domain of κ and γ from some mammals (humans, mouse, rabbit, rat, and pig) and of the L chains from *Gallus*, *Xenopus, Rana, Heterodontus, Carcharhinus*, and the two teleosts, were aligned with the Pileup programme (Devereux et al. 1984) and analyzed with the PAUP software (Swofford 1991). Several evolutionary trees were obtained (data not shown) and in all of them the L chain of Atlantic cod and rainbow trout were placed at the root i.e., neither could be classified as κ nor λ.



Variable domain. One clone of Atlantic cod contains the main part (except FR1) of the V_L domain and three clones from rainbow trout have a complete V_L. These three VLs are very similar in FR2 and FR3 but cannot be considered as belonging to the same family, due to the great variation of the amino acid sequence in FR1 and CDR1 (Fig. 8). In FR1 only approximately 50% of the amino acids are identical between the three clones. A comparison of FR2, FR3, and FR4 between cod and rainbow trout shows 10/15, 20/32, and 7/12 amino acid identities. The V_L segment of rainbow trout is most similar to those of mammals (mouse and human; Kabat et al. 1991), regarding both the number and the identities of the amino acids of the different regions (data not shown). The four frameworks show 30%–50%, 70%–85%, 50%–65% and 40%–60% identity with the different V_L families of mammals, but no difference between κ and γ was noticed. In a recent paper by Zezza and co-workers (1992) V_L sequences from the *Xenopus* ρ chain have been studied and a phylogenetic tree is presented indicating that *Xenopus* V_L ρ is of the κ type, while the σ seems to form its own branch in the phylogenetic tree. A similar analysis with the V_L framework regions including the *Gadus* and *Oncorhynchus* gives a result shown in Figure 9. Although the relative length differs from that of *Xenopus* σ, the V_L of the teleosts

Fig. 6. Sequence alignment of the constant regions of IgL from different species to clone cSg L2A from Atlantic cod (*Gadus morhua*) and clone rtSg E from rainbow trout (*Oncorhynchus mykiss*). The alignment was performed with the use of the conserved cystein and tryptophan residues, which are indicated in *bold print* in all the sequences. Identical amino acids between the Atlantic cod and rainbow trout are indicated by a *bar* between the sequences. In the other sequences, identity with both these two species is indicated by a *bold dot*, identity with Atlantic cod by a *lower stop* sign, and with rainbow trout by an *upper stop* sign. Data on the horned shark (*Heterodontus francisci*) are from Shamblott and Litman (1989a), on the sandbar shark (*Carcharihnus plumbeus*) from Schluter and co-workers (1989), on the clawed frog (*Xenopus laevis*) from Schwager and co-workers (1991) and Zezza and co-workers (1991), on the bullfrog (*Rana catesbeiana*) from Mikoryak and Steiner (1988), on the chicken (*Gallus gallus domesticus*) from Reynaud and co-workers (1987), and on the mouse (*Mus musculus*) and humans (*Homo sapiens*) from Kabat and co-workers (1991). The amino acids are numbered according to Kabat and co-workers (1991).

Table 1. Comparison of numbers and percentages of amino acid identities of the constant part of immunoglobulin light chain from Atlantic cod (*Gadus morhua*) and rainbow trout (*Oncorhynchys mykiss*) with those of other species.*

Species-isotype		Total (N)	Atlantic cod		Rainbow trout		Both species	
			n	%	n	%	n	%
Gadus m.		107	–	–	56	54.9	–	–
Oncorhynchus m.		102	56	54.9	–	–	–	–
Heterodontus f.		108	29	27.1	28	27.4	20	19.6
Carcharhinus p.		107	31	29.0	26	25.5	20	19.6
Xenopus l.	ρ	107	30	28.0	28	27.4	21	20.6
	σ	106	25	23.6	28	27.4	19	18.6
Rana c.		108	26	24.3	23	22.5	16	15.7
Gallus d.		105	29	27.6	33	32.4	22	21.6
Mus m.	κ	106	35	33.0	35	34.3	24	23.5
Homo s.	κ	106	34	32.1	34	33.3	23	22.5
Mus m.	λ	105	31	29.5	38	37.2	24	23.5
Homo s.	λ	105	31	29.5	30	29.4	21	20.6

* The estimates derive from the alignment shown in Figure 6.
N Total number of amino acids.
n Number of amino acids indentical to either Atlantic cod, rainbow trout, or both species.

seem to form their own branch between the κ and γ isotypes.

Discussion

The finding that the *IgL* locus of teleost fish has an organization similar to an elasmobranch type (or a very complicated λ type of mammal) shows that the gene duplications within the heavy and light chain loci have followed different pathways in evolution. In most other vertebrate groups the organization of the genes for the two chains are of the same type (see introduction). In bony fish, however, the H-chain locus is organized as in mammals and amphibians (Amemiya and Litman 1990; Wilson et al. 1990; Bengtén et al. 1991) but the L-chain locus is as in elasmobranchs. This implies that the two different pathways for gene duplication of *Ig* genes were present in an ancestor of elasmobranch and teleost fish. Rudiments of the "shark" pathway can still be found in the λ locus of mouse, rabbits, and humans, and perhaps in teleost fish. In ruminants and horses >90% of the light chain is of γ isotype. Although the organization of their L-chain loci are unknown, it seems probable that their λ locus has an organization with many *VL* segments, as indicated in sheep (Reynaud et al. 1991). Thus, it seems that the gene duplication pathway is not isotype specific. Our results also support this, as the L chain in horned and sandbar sharks is most related to the λ isotype (Shamblot and Litman 1989; Hohman et al. 1992) and the L chain from Atlantic cod and rainbow trout is not more related to the κ than to the λ isotype (Fig. 9). However, the nonamer/heptamer sequences are separated by 23 nucleotides in clone rtSg 4 and rtSg 9, favoring the κ type in at least the rainbow trout (Max 1989; Fig. 2). In rainbow trout the amino acid sequences of clone rtSg E and rtSg 9 are more equal to each other than to the others, including the lack of the terminal Ser-Gly residues (Fig. 8), and in that they are missing the 73 nt in the 3' non-coding part of the cDNA (Fig. 2). This might indicate the start of a divergence into two isotypes of the light chain in teleost fish. The observation of two L-chain isotypes in channel catfish (Lobb et al. 1984) was based on the binding of monoclonal antibodies, but, since monoclonal antibodies can differentiate among only a very small number of amino acids in an epitope, it seems likely that a phenomenon exists in channel catfish similar to that in rainbow trout.

An intriguing finding is that the constant part of the *IgL* gene is transcribed before rearrangement and often spliced to the *J* segment (Figs. 1, 2). This might be explained in two ways. Firstly, similar findings have been reported in mammalian pre-B-cells and cell lines for both IgL (Van Ness et al. 1981; Nelson et al. 1985) and IgH (Alt et al. 1982) and is considered to be a prerequisite for the initiation of rearrangement (Staudt and Lenardo 1991). In agreement with this we find more unrearranged transcripts in the main hematopoietic organ, the head kidney (reviewed by Rowley et al. 1988), but such transcripts also occur in the spleen of the two fish species. This indicates that the spleen also

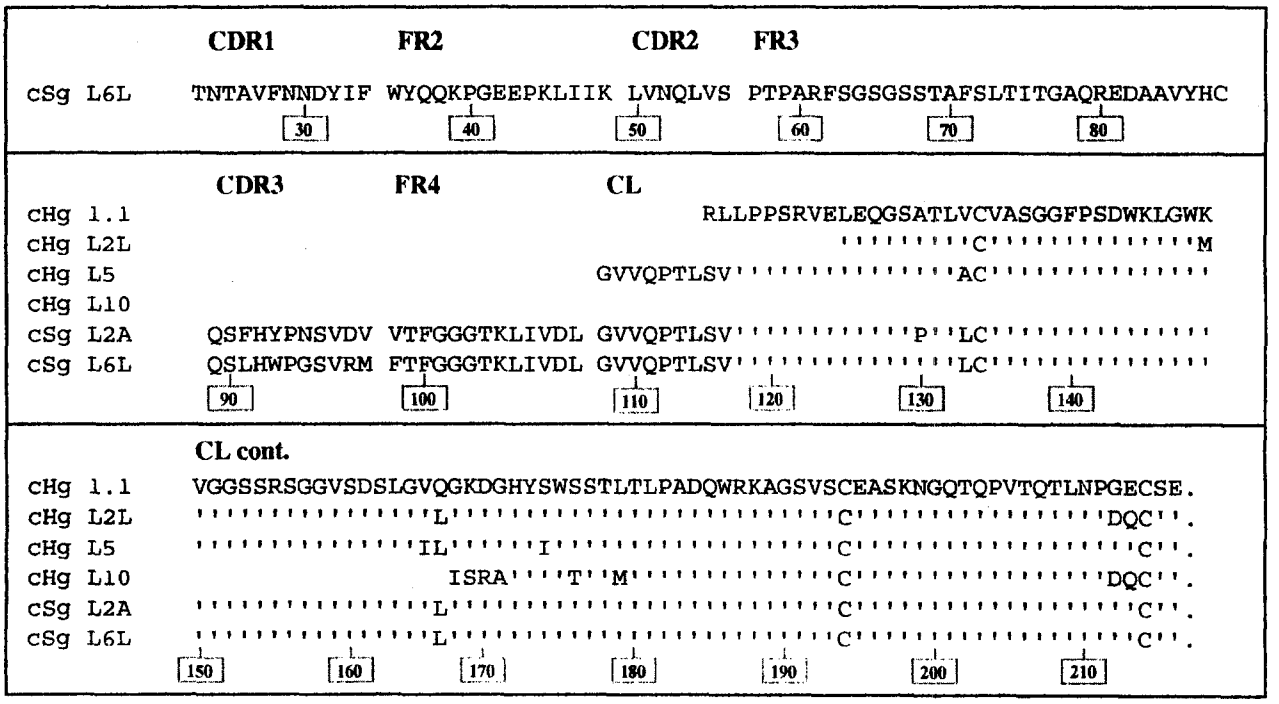


Fig. 7. Alignment of the deduced amino acids from the six IgL chain clones from two cDNA libraries from Atlantic cod (*Gadus morhua*). The single-letter code for amino acids is used and identities are indicated by *apostrophe signs*, except for conserved cysteine residues and stop codons, which are indicated by a *full stop*. The numbering of the amino acids was performed according to Kabat and co-workers (1991).

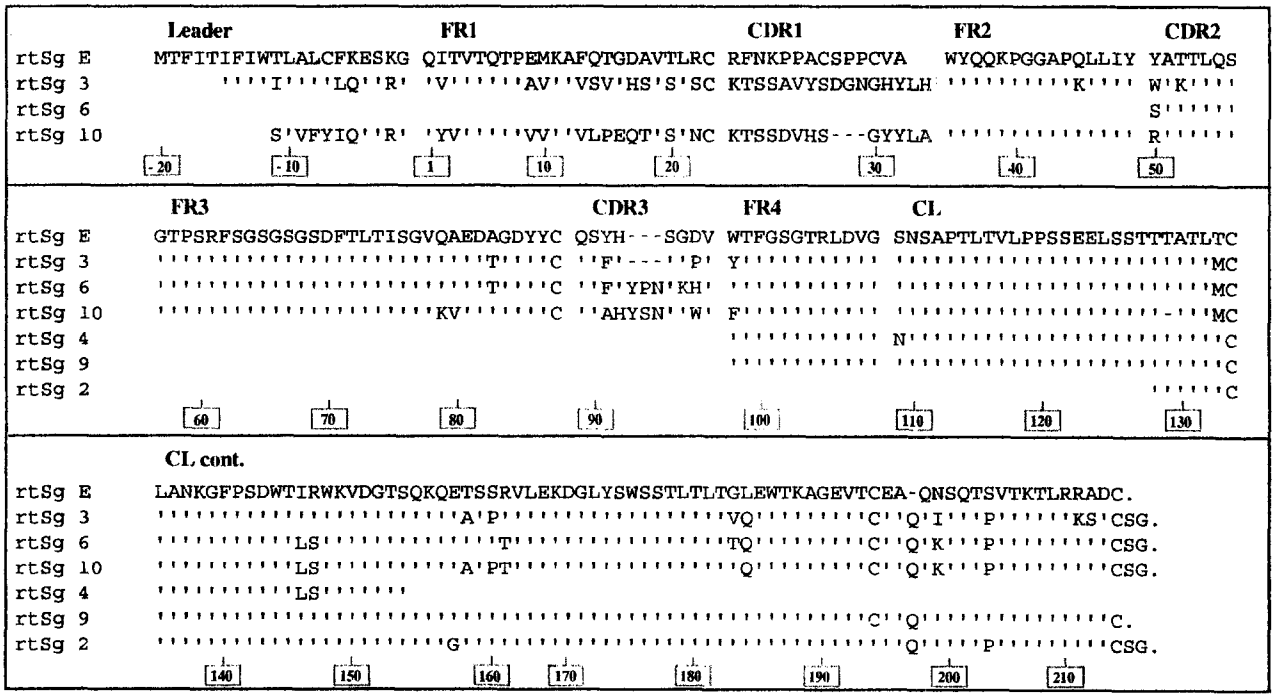


Fig. 8. Alignment of the deduced amino acid of seven IgL chain clones from a spleen cDNA library of rainbow trout (*Oncorhynchus mykiss*). The single-letter code for amino acids is used. *Apostrophes* indicate identity to clone rtSg E and *dashes* indicate gaps introduced to optimize the alignment. The numbering of the amino acids was performed according to Kabat and co-workers (1991).

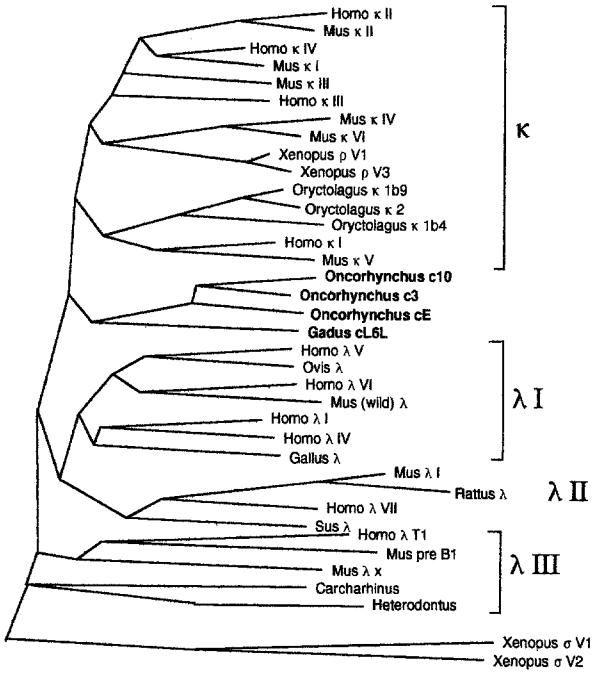


Fig. 9. Unrooted phylogenetic tree of V_L region framework sequences. The tree was constructed with the MegAlign programme (DNAStar) according to the Clustal procedure (Higgins and Sharp 1989). Analysis using the procedure of Hein (1990) gives essentially the same tree. The amino acid sequences were obtained from Kabat and co-workers (1991) and Zezza and co-workers (1992).

may harbour pre-B-lymphocytes i.e., also function as a lymphopoietic organ. A second explanation of this phenomenon is the finding by Wang and Calame (1985) that immunoglobulin enhancers are able to activate transcription of V_H promoters located as far as 17.5 kb away from the gene. Thus, with an organization of the type found in the L-chain locus of the two teleost species, a functional rearrangement and activation of transcription may activate transcription of other promoters of nonrearranged clusters. Quantitatively, the Atlantic cod have more unrearranged mRNA in the two organs than rainbow trout – the amounts being so high that we have had difficulties finding a rearranged clone containing a V_L segment from the cDNA libraries of this species. The fact that the locus of this species seems more complex than that of the rainbow trout (Figs. 3, 4) gives a little support to the latter explanation.

In conclusion, we have demonstrated that the loci for the IgL chain in Atlantic cod and rainbow trout have a complex organization which is most similar to those of the two Ig chains in elasmobranch fish. Transcription of the loci takes place before rearrangement/splicing, as is the case for mammalian pre-B-cells. The IgL chain of the two teleost species can be classified neither as the κ nor the λ isotype in mammals, nor as any other isotype described in other species.

Acknowledgments. We acknowledge the skilfull technical assistance of Siv Strömberg. We would like to thank Professor Thomas Leanderson and Professor Jan Andersson for valuable comments on the manuscript, and the latter for providing excellent working facilities. We thank Dr. Ivar Hordvik, Bergen, Norway, for probes of IgH from Atlantic salmon and Dr. Ann Thuvander for the supply of antiserum. This work was supported by grants from The Swedish Forestry and Agricultural Council (SJFR no. 42.0053/90), the BRIDGE Programme (Project no. PL890267) of the European Community, and by contributions from Stiftelsen Lars Hiertas minne, Ahlstrands zoologiska gåvomedel (the Swedish Royal Academy of Sciences), and Inez Johanssons fond (Uppsala University).

References

Alt, F. W., Rosenberg, N., Enea, V., Siden, E., and Baltimore, D. Multiple immunoglobulin heavy-chain transcripts in Abelson murine leukemia virus-transformed lymphoid cell lines. *Mol Cell Biol 2*: 386–400, 1982

Amemiya, C. T. and Litman, G. W. Complete nucleotide sequence of an immunoglobulin heavy-chain gene and analysis of immunoglobulin gene organization in a primitive teleost species. *Proc Natl Acad Sci USA 87*: 811–815, 1990

Bengtén, E., Leanderson, T., and Pilström, L. Immunoglobulin heavy chain cDNA from the teleost Atlantic cod (*Gadus morhua* L.): nucleotide sequences of secretory and membrane form show an unusual splicing pattern. *Eur J Immunol 21*: 3027–3033, 1991

Church, G. M. and Gilbert, W. Genomic sequencing. *Proc Natl Acad Sci USA 81*: 1991–1995, 1984

Devereux, J., Haeberli, P., and Smithies, O. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res 12*: 387–395, 1984

Earley, P., Rogers, J., Davies, M., Calame, K., and Hood, L. An immunoglobulin heavy chain variable region gene is generated from three segments of DNA: V_H, D and J_H. *Cell 19*: 981–992, 1980

Feinberg, A. P. and Vogelstein, B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem 132*: 6–13, 1983

Harding, F. A., Cohen, N., and Litman, G. W. Immunoglobulin heavy chain gene organization and complexity in the skate, *Raja erinacea*. *Nucleic Acids Res 18*: 1015–1020, 1990

Harlow, E. and Lane, D. *Antibodies – A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, 1988*

Hayzer, D. J. Immunoglobulin lambda light chain evolution: Igl and Igl-like sequences form three major groups. *Immunogenetics 32*: 157–174, 1990

Hein J. Unified approach to alignment and phylogenetics. *Methods Enzymol 183*: 626–645, 1990

Higgins, D. G. and Sharp, P. M. Fast and sensitive multiple sequence alignments on a microcomputer. *CABIOS 5*: 151–153, 1989

Hohman, V. S., Schluter, S. F., and Marchalonis, J. J. Complete sequence of a cDNA clone specifying sandbar shark immunoglobulin light chain: Gene organisation and implications for the evolution of light chains. *Proc Natl Acad Sci USA 89*: 276–280, 1992

Hordvik, I., Voie, A.-M., Glette, J., Male, R., and Endresen, C. Cloning and sequence analysis of two isotypic IgM heavy chain genes from Atlantic salmon, *Salmo salar* L. *Eur J Immunol 22*: 2957–2962, 1992

Huynh, T. V., Young, R. A., and Davies, R. W. Costructing and screening cDNA libraries in λgt10 and λgt11. *In* D. M. Glower (ed.): *DNA Cloning – A Practical Approach, vol. 1., pp. 49–78*, IRL Press, Oxford, 1985

Israelsson, O., Petersson, A., Bengtén, E., Wiersma, E. J., Andersson, J., Gezelius, G., and Pilström, L. Immunoglobulin concentration in Atlantic cod, *Gadus morhua* L., serum and crossreactivity between anti-cod-antibodies and immunoglobulin from other species. *J Fish Biol 39*: 265–278, 1991

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 Institutes of Health, 1991

Kawakami, T., Takahashi, N., and Honjo, T. Complete nucleotide sequence of mouse immunoglobulin μ gene and comparison with other immunoglobulin heavy chain genes. *Nucleic Acids Res 8*: 3933–3945, 1980

Kokubu, F., Hinds, K., Litman, B., Shamblott, M. J., and Litman, G. W. Complete structure and organization of immunoglobulin heavy chain constant region genes in a phylogenetically primitive vertebrate. *EMBO J 7*: 1979–1988, 1988

Lee, M. A., Bengtén, E., Daggfeldt, A., Rytting, A.-S., and Pilström, L. Characterisation of rainbow trout cDNAs encoding a secreted and membrane-bound Ig heavy chain and the genomic intron upstream of the first constant exon. *Mol Immunol*, in press, 1993

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

Max, E. E. Immunoglobulins: molecular genetics. *In* W. E. Paul, (ed.): *Fundamental Immunology, 2nd edn.*, pp. 235–290, Raven Press, New York, 1989

Mikoryak, C. A. and Steiner, L. A. Amino acid sequence of constant region of immunoglobulin light chains from *Rana catesbelana*. *Mol Immunol 25*: 695–703, 1988

Nelson, K. J., Kelley, D. E., and Perry, P. P. Inducible transcription of the unrearranged κ constant region locus is a common feature of pre-B cells and does not require DNA or protein synthesis. *Proc Natl Acad Sci USA 82*: 5305–5309, 1985

Pilström, L. and Petersson, A. Isolation and partial characterization of immunoglobulin from cod (*Gadus morhua* L.). *Dev Comp Immunol 15*: 142–152, 1991

Reynaud, C.-A., Anquez, V., Grimal, H., and Weill, J.-C. A hyperconversion mechanism generates the chicken light chain preimmune repertoire. *Cell 48*: 379–388, 1987

Reynaud, C.-A., Dahan, A., Anquez, V., and Weill, J.-C. Somatic hyperconversion diversifies the single V_H gene of the chicken with a high incidence in the D Region. *Cell 59*: 171–183, 1989

Reynaud, C.-A., Mackay, C. R., Müller, R. G., and Weill, J.-C. Somatic generation of diversity in a mammalian primary lymphoid organ: the sheep ileal peyer's patches. *Cell 64*: 995–1005, 1991

Rowley, A. F., Hunt, T. C., Page, M., and Mainwarning, G. Fish. *In* A. F. Rowley and N. A. Ratcliffe (eds.): *Vertebrate Blood Cells*, pp. 19–128, Cambridge University Press, Cambridge, 1988

Saluk, P. H., Krauss, J., and Clem, L. W. The presence of two antigenetically distinct light chains (κ and γ?) in alligator immunoglobulins. *Proc Soc Exp Biol Med 133*: 365–369, 1970

Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular Cloning. A Laboratory Manual, 2nd edn.*, Cold Spring Harbor Laboratory, Cold Spring Harbor, 1989

Sanger, F., Miklen, S., and Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA 74*: 5463–5467, 1977

Schluter, S. F., Hohman, V. S., Edmundson, A. B., and Marchalonis, J. J. Evolution of immunoglobulin light chains: cDNA clones specifying sandbar shark constant regions. *Proc Natl Acad Sci USA 86*: 9961–9965, 1989

Schwager, J., Grossberger, D., and Du Pasquier, L. Organization and rearrangement of immunoglobulin *M* genes in the amphibian *Xenopus*. *EMBO J 7*: 2409–2415, 1988

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

Selsing, E., Durdik, J., Moore, M. W., and Persiani, D. M. Immunoglobulin λ Genes. *In* T. Honjo, F. W. Alt, and T. H. Rabbitts (eds.): *Immunoglobulin Genes*, pp. 111–122, Academic Press, London, 1989

Senapathy, P., Shapiro, M. B., and Harris, N. L. Splice junctions, branch point sites and exons: sequence statistics, identification and application to genome project. *Methods in Enzymology 183*: 252–272, 1990

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, 1989a

Shamblott, M. J. and Litman, G. W. Complete nucleotide sequence of primitive vertebrate immunoglobulin light chain genes. *Proc Natl Acad Sci USA 86*: 4684–4688, 1989b

Staudt, L. M. and Lenardo, M. J. Immunoglobulin gene transcription. *Annu Rev Immunol 9*: 373–378, 1991

Swofford, D. L. *PAUP – Phylogeny Analysis Using Parsimony, Version 3.0s.* Computer programme distributed by the Illinois Natural History Survey, Champaign, Illinois, 1991

Thuvander, A., Hongslo, T., Jansson, E., and Sundquist, B. Duration of protective immunity and antibody titres measured by ELISA after vaccination of rainbow trout, *Salmo gairdneri* Richardson, against vibriosis. *J Fish Disease 10*: 479–486, 1987

Van Ness, B. G., Weigert, M., Coleclough, C., Mather, E. L., Kelley, D. E., and Perry, R. P. Transcription of the unrearranged mouse *C*κ locus: sequence of the initiation region and comparison of activity with a rearranged *V*κ-*C*κ gene. *Cell 27*: 593–602, 1981

Wang, X.-F. and Calame, K. The endogenous immunoglobulin heavy chain enhancer can activate tandem V_H promoters separated by a large distance. *Cell 43*: 659–665, 1985

Wilson, M. R., Marcus, A., van Ginkel, F., Miller, N. W., Clem, L. W., Middleton, D., and Warr, G. W. The immunoglobulin M heavy chain constant region gene of the channel catfish, *Ictalurus punctatus*: an unusual mRNA splice pattern produces the membrane form of the molecule. *Nucleic Acids Res 18*: 5227–5233, 1990

Zachau, H. Immunoglobulin light-chain genes of the κ type in man and mouse. *In* T. Honjo, F. W. Alt, and T. H. Rabbitts (eds.): *Immunoglobulin Genes*, pp. 92–109, Academic Press, London, 1989

Zezza, D. J., Mikoryak, C. D., Schwager, J., and Steiner, L. A. Sequence of C region of L chain from *Xenopus laevis* Ig. *J Immunol 146*: 4041–4047, 1991

Zezza, D. J., Stewart, S. E., and Steiner, L. A. Genes encoding *Xenopus laevis* Ig L chains. Implications for the evolution of κ and γ chains. *J Immunol 149*: 3968–3977, 1992