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Genomic organization and sequences of immunoglobulin light chain genes in a primitive vertebrate suggest coevolution of immunoglobulin gene organization

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The genomic organization and sequence of immunoglobulin light chain genes in *Heterodontus francisci* (horned shark), a phylogenetically primitive vertebrate, have been characterized. Light chain variable (V_L) and joining (J_L) segments are separated by 380 nucleotides and together with the single constant region exon (C_L), occupy <2.7 kb, the closest linkage described thus far for a rearranging gene system. The V_L segment is flanked by a characteristic recombination signal sequence possessing a 12 nucleotide spacer; the recombination signal sequence flanking the J_L segment is 23 nucleotides. The V_L genes, unlike heavy chain genes, possess a typical upstream regulatory octamer as well as conserved enhancer core sequences in the intervening sequence separating J_L and C_L. Restriction mapping and genomic Southern blotting are consistent with the presence of multiple light chain gene clusters. There appear to be considerably fewer light than heavy chain genes. Heavy and light chain clusters show no evidence of genomic linkage using field inversion gel electrophoresis. The findings of major differences in the organization and functional rearrangement properties of immunoglobulin genes in species representing different levels of vertebrate evolution, but consistent similarity in the organization of heavy and light chain genes within a species, suggests that these systems may be coevolving.

Key words: coevolution/field inversion gel electrophoresis/immunoglobulin/recombination signal sequence/regulatory octamer/V_L gene organization

Introduction

Antibody diversity is generated during the somatic development of B lymphocytes through rearrangement of chromosomally separated segmental elements (Tonegawa, 1983; Blackwell and Alt, 1988). Although the structures of the segmental elements and the mechanism of somatic reorganization are similar in species as phylogenetically distant as the earliest representative jawed vertebrates, dramatic changes in the organization and presumably, regulation of immunoglobulin genes have occurred. In *Heterodontus*, a primitive elasmobranch, immunoglobulin heavy chain variable (V_H), diversity (D_H) and joining (J_H) segments are linked closely to constant region (C_H) exons and are arrayed in multiple, individual ~16 kb clusters (Hinds and Litman, 1986; Kokubu *et al.*, 1988a,b). In ~50% of these germline clusters, segmental elements are joined; however, it is not understood whether these joined genes are functional. Although the organization of the C_H exons and the differential RNA splicing mechanism that regulates the transcription of secretory versus transmembrane immunoglobulin forms are remarkably similar between *Heterodontus* and higher vertebrates (Kokubu *et al.*, 1988b; Peterson and Perry, 1989), the close linkage of segmental elements and presence of two D segments within an individual heavy chain cluster are more typical of T cell antigen receptors (TCR) (Kokubu *et al.*, 1988a). Furthermore, both *Heterodontus* heavy chain and TCR genes lack the regulatory octamer found upstream of the transcription start site of immunoglobulin heavy and light chain genes of all higher vertebrates (Kokubu *et al.*, 1988a).

N-terminal sequences of *Heterodontus* immunoglobulin light chains have been reported (Kehoe *et al.*, 1978) and recently *Heterodontus* immunoglobulin light chain genes have been identified by antibody screening of a spleen cDNA library (Shamblott and Litman, 1989). The predicted amino acid sequences of *Heterodontus* immunoglobulin light chain constant regions (C_L) are most related to lambda light chains of higher vertebrates; however, the DNA sequences of the putative coding regions of these primitive light chains are related most to β-TCR (Shamblott and Litman, 1989). In order to characterize further the evolution of antigen receptors, light chain genes have been isolated from a *Heterodontus* genomic DNA library and their organization, structure and genomic complexity are described.

Results

Linkage of V_L, J_L and C_L segments

A *Heterodontus francisci* (horned shark) genomic DNA library was screened with HFL, a homologous light chain cDNA probe (Shamblott and Litman, 1989). Positive clones were isolated and mapped both by conventional techniques using segment-specific probes (HFV_L, HSLJ, HFC_L), and by estimating linkage distances from the lengths of fragments generated by the polymerase chain reaction (PCR) using primers that complement the 5′ portion of the light chain variable (V_L) and 3′ portion of the C_L regions (Figure 1a). Based on the sizes of the PCR products and the complete DNA sequence analyses of two genes (see below), V_L, light chain joining (J_L) and C_L segments are linked closely (<2.7 kb). At least 40 unique genomic light chain clusters have been identified.

Light chain gene complexity

Southern blot analysis of *Heterodontus* genomic DNA digested with *Eco*RI (Figure 1b) indicates a significant degree of complexity in the hybridization pattern that has been confirmed in analyses using other restriction endonucleases (not illustrated). Fewer hybridizing fragments are apparent than were noted in similar analyses with a V_H-specific probe (HFV_H) (Litman *et al.*, 1985a). Since a full copy length, V_L-J_L-C_L (HFL) probe was used, some of the

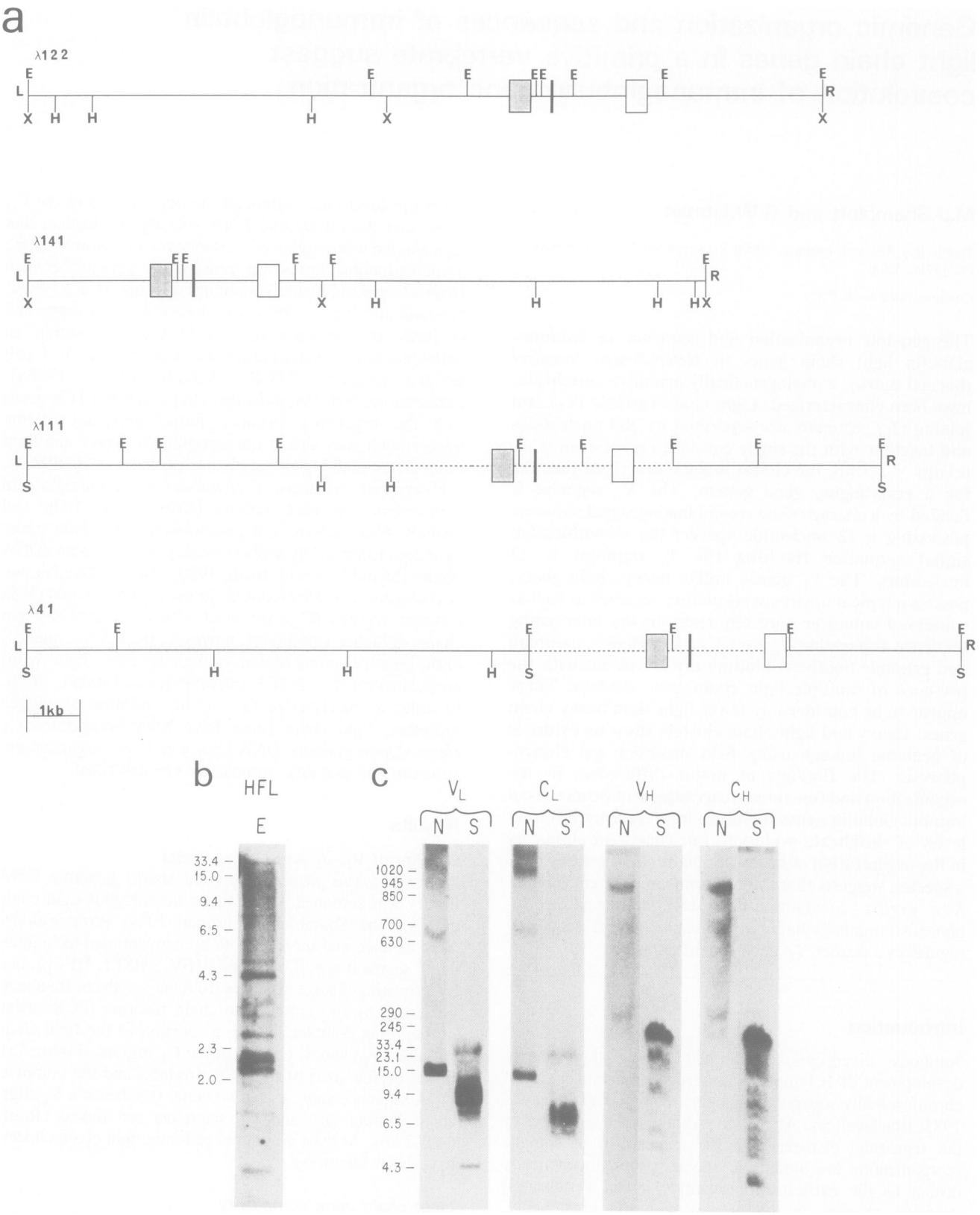

Fig. 1. (**a**) Partial restriction maps (±100 nt) of light chain gene clones. Left (L) and right (R) are λ vector arms. V_L ⊠, J_L ▮ and C_L □ assignments are based on complete nucleotide sequences (λ122 and λ141) and are absolute; relative positions of hybridizing sequences in λ111 and λ41 are shown by an interrupted line. (**b**) Southern blot analysis of *Eco*RI (E)-digested *Heterodontus francisci* genomic DNA (10 μg), transferred to Zeta-Probe™ (BioRad) and hybridized (Litman *et al.*, 1985) with HFL (Shamblott and Litman, 1989). Probe specific activity was 2×10^9 c.p.m./μg; 5×10^5 c.p.m./ml and 96 h autoradiographic exposure. Standard values in kb pairs are for λ DNA digested with *Xho*I and *Hin*dIII. (**c**) Southern blot analysis of *Heterodontus* erythrocyte DNA embedded in agarose and digested with *Not*I (N) or *Sst*I (S), separated by field inversion gel electrophoresis (Carle *et al.*, 1986), transferred to Duralon-UV™ (Stratagene) and hybridized with HFV_L (V_L), HFC_L (C_L), HFV_H (V_H) or C_H1_{801} (C_H). Probe specific activities were: HFV_L and HFC_L, 9.6×10^8 c.p.m./μg and 2.4×10^5 c.p.m./ml; and V_H and C_H, 2.4×10^9 c.p.m./μg and 6×10^5 c.p.m./ml, 96 h autoradiographic exposure. Standard values are for *S.cerevisiae* chromosomes (Bio-Rad) and as in (b).

complexity in the genomic digestion pattern could arise from internal *Eco*RI site(s) present in at least some light chain gene clusters (Figure 1a).

In order to estimate the relative numbers of light and heavy chain genes more precisely, replica lifts of a genomic library were screened in parallel with C_L (HFC_L)- and C_H (C_H1_{801})-specific probes. At identical, moderate stringency hybridization-wash conditions, the light chain probe detects only 20% as many clones as hybridize with the heavy chain-specific probe. Restriction mapping of representative isolates confirms the unique identity of individual hybridizing components. It is unlikely that restrictions in probe specificity account for this large difference, since C_L genes appear to be more conserved than the region of heavy chain genes complemented by the C_H-specific probe used in the parallel analyses (Kokubu *et al.*, 1987). Since all phage clones characterized thus far contain only a single V_L-J_L-C_L cluster, the paucity of C_L hybridizing clones cannot be explained by the presence of multiple clusters in a single phage clone (see below). Under-representation of light chain genes in the genomic library could explain these results and is difficult to ascertain; however, the genomic blotting studies discussed above also suggest fewer light chain clones. Finally, all C_L^+ clones also hybridize with HFV_L. Unless a large family of light chain genes is present that is unrelated to V_L and C_L, it is most reasonable to conclude that greater numbers of heavy chain gene clusters are present.

Extended linkage

In order to examine extended linkage of light chain genes and identify possible linkage between heavy and light chain gene loci, Southern blot analyses were carried out using field inversion gel electrophoresis (FIGE) of restriction endonuclease-digested *Heterodontus* erythrocyte DNA (Figure 1c). With *Not*I digests, HFV_L- and HFC_L-specific probes hybridize to the same fragments, consistent with the close linkage of V_L and C_L segments described above. With *Sst*I digests, hybridization is confined largely to two different major fragments, consistent with cleavage between V_L and C_L segments. For comparison, the results of parallel studies with HFV_H (Litman *et al.*, 1985a) and C_H1_{801} (Kokubu *et al.*, 1987) probes are shown. The similar hybridization patterns of V_H- and C_H-specific probes with *Not*I-digested DNA and with a major >33.4 kb fragment(s) produced by digestion with *Sst*I are consistent with the known linkage patterns of *Heterodontus* heavy chain genes (Kokubu *et al.*, 1988a). The failure to detect similarities in the hybridization patterns with heavy and light chain-specific probes suggests that these gene families are not linked closely. In additional experiments, ~100 V_L^+ genomic clones have been hybridized with a C_L probe; only two $V_L^+C_L^-$ clones were detected. When these clones were restriction mapped, V_L hybridization was found to map to the right arm of λ, i.e. the absence of hybridization with C_L can be accounted for by the mechanics of λ cloning. From this experiment, however, it is possible to conclude that another gene cluster does not occur within ~13–15 kb 5′ of these V_L segments.

Complete nucleotide sequence of Heterodontus immunoglobulin light chain genes

The complete nucleotide (nt) sequences of genomic light chain genes λ122 and λ141 are illustrated in Figure 2. It is apparent that the 5′ and 3′ untranslated segments, as well as the intervening sequences separating V_L-J_L and J_L-C_L, are related closely. The intervening sequences that divide the leader region also are related closely, with the exception of nine fewer nucleotides in λ141. The predicted amino acid sequences of the leader regions are identical and are only 16 residues, shorter than the leader sequences found in higher vertebrate immunoglobulin genes. Nucleotide differences were not found in framework regions (FR1 and FR3); of the three nt differences that occur in FR2, only one results in a replacement substitution. All nt changes in the complementarity determining regions (CDR), are replacement substitutions; two amino acid changes occur in each CDR segment. Identical recombination signal sequences (RSS) (including the spacer segments) are located 3′ of V_L and 5′ of J_L. The 12 nt spacer associated with V_L and the 23 nt spacer associated with J_L are characteristic of mammalian κ light chain genes (Tonegawa, 1983). V_L and J_L segments, including the RSS, are separated by only 316 nt. When these two genes are compared, the predicted coding regions of J_L and C_L are identical. A typical light chain-type regulatory octamer is located 134 nt 5′ of the initiation codon (Parslow *et al.*, 1984; Falkner *et al.*, 1986). Short DNA segments matching consensus immunoglobulin enhancer sequences are located within the J_L-C_L intervening sequence; however, their spacing differs from that associated with mammalian immunoglobulin light chains (Church *et al.*, 1985). A number of extended base repeats are noted in the intervening sequences separating J_L and C_L. A polyadenylation signal sequence is located 329 nt 3′ of the termination codon.

Germline joined light chain gene clusters are not found

In the *Heterodontus* heavy chain gene system, ~50% of the immunoglobulin gene clusters are V_HD_H- or $V_HD_HJ_H$-joined in the germline of non-lymphoid cells. In order to evaluate this possibility in the light chain gene system, a PCR-generated probe complementing the highly conserved V_L-J_L intervening sequence (122IVS) was used to screen >100 $V_L^+C_L^+$ genomic phage clones. The probe hybridizes at essentially equivalent intensity to all isolates, a finding that is consistent with the presence of closely related intervening sequences in each clone. The absence of germline joining is documented further by the restriction maps of the four clones shown (Figure 1a) as well as by the partial maps of additional clones that were characterized in the course of these studies. It is unlikely that any V_L and J_L segments that belong to the light chain multigene family detected by these probes are joined in the germline.

Segmental joining

Relating a rearranged immunoglobulin gene (cDNA) to a specific germline gene is difficult with closely related members of a multigene family (Kokubu *et al.*, 1988a). Only two complete germline and two full copy length cDNA sequences of light chains are available for comparison. The sequence of λ122 differs from cDNA 1501 (Shamblott and Litman, 1989) by 3 nt in CDR2, a single nucleotide in C_L and 4 nt in a 312 nt segment of the 3′ untranslated region. The differences between the sequences of genomic clone λ122 and cDNA 1501 could be isotypic, allelic or somatic. It is noted that a G occurs in the constant region at position

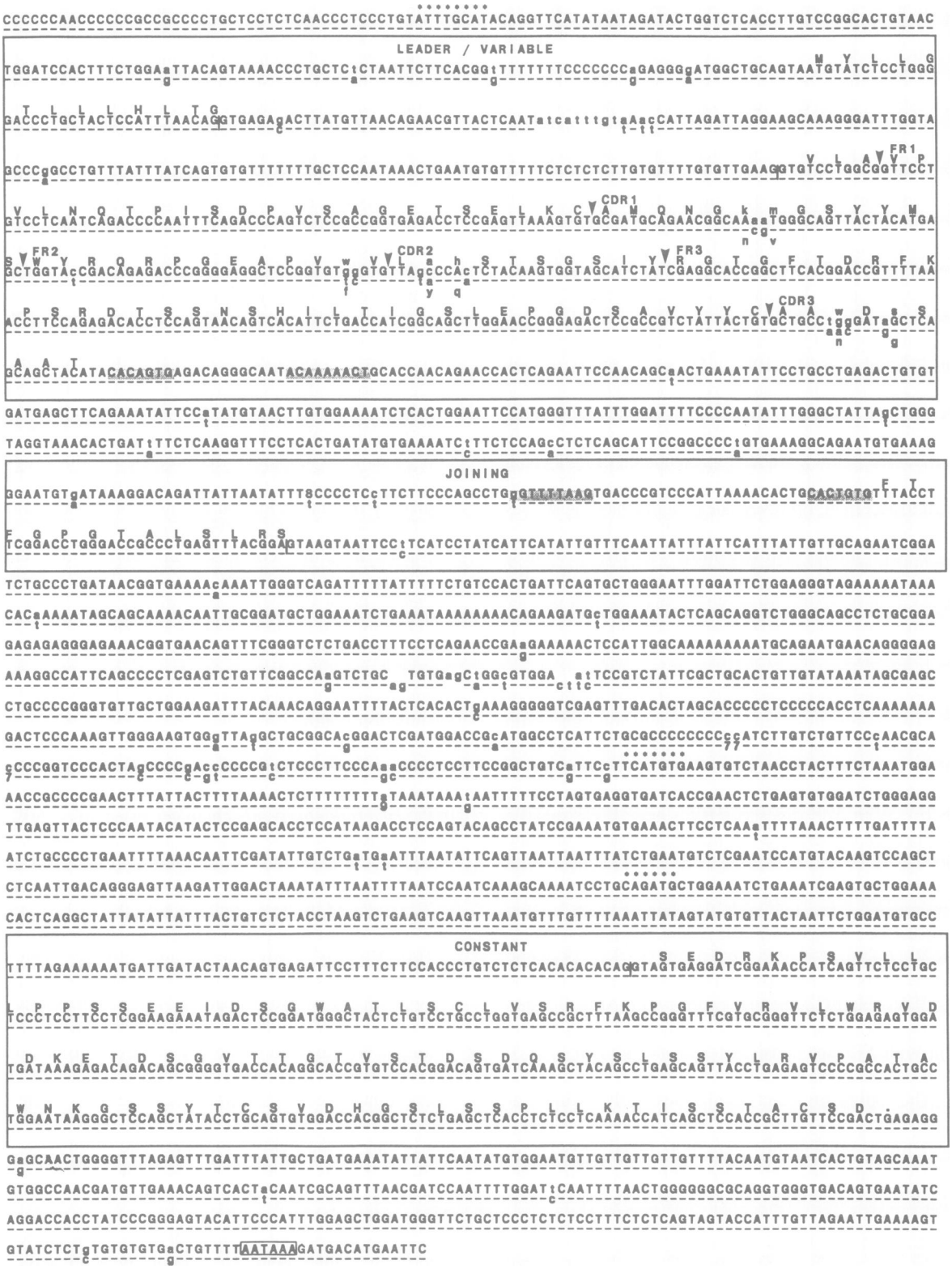

Fig. 2. Complete nucleotide sequences of *Heterodontus* genomic light chain genes λ122 above, EMBL/GenBank accession X15316 and λ141 below, EMBL/GenBank accession X15315, shown in 100 nt blocks. Identities between the two sequences are shown by (−), differences indicated by lower-case nucleotides. At four intervening sequence positions, uncertainty in the number (±1−2) of contiguous identical nucleotides is indicated by Stanford (Intelligenetics) ambiguity codes. Predicted coding amino acids in one-letter code above λ122 and where different, in lower-case and shown below λ141. Stop codon (.), leader, V_L, J_L and C_L boundaries are from cDNA analyses (Shamblott and Litman, 1989, and unpublished observations); V_L and J_L recombination signal sequences are shaded. The 5′ boundaries of J_L and C_L differ from those that were assigned by interspecies DNA homologies (Shamblott and Litman, 1989). The putative regulatory octamer and enhancer-like sequences in the intervening sequence separating J_L and C_L are noted by (*) above; a putative polyadenylation signal sequence is enclosed. Sequences were determined on both strands (Sanger *et al.*, 1977).

2660 of λ122 and λ141 (Figure 2); in both cDNAs, a C is present at this position, resulting in an amino acid replacement (Shamblott and Litman, 1989).

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                    VL (CDR3)                          JL
CONSENSUS     GATAGCTCAGCAGctacata ttTACCTTCGGACCTGGGAC
GERMLINE
cDNA 1501     GATAGCTCAGCAGGCTACTTAGGTACCTTCGGACCTGGGAC
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In the V_L (CDR3) and J_L regions shown above, sequences of λ122 and λ141 are identical. The predicted deletion of nucleotides in the germline sequence is indicated by italicized lower-case letters. The RSSs associated with the 3′ of V_L and 5′ of J_L (Figure 2) are not shown. The sequence of cDNA 1501 through the VJ join is highlighted by shading, representing nucleotides that are shared with the consensus germline segments or by a reverse image delineating nucleotides that do not occur in the consensus and may represent template-independent additions (Alt and Baltimore, 1982). While it appears that both the addition and deletion of nucleotides occurs at the V−J junctions, unequivocal statements regarding immunoglobulin gene segmental joining and other somatic changes requires the identification of the parent germline clusters.

Discussion

The results reported here represent the first descriptions of the genomic structure and organization of light chain genes in a species below the phylogenetic level of the avians (Reynaud *et al.*, 1985). The identification of multiple light chain genomic clones that contain closely linked V_L, J_L and C_L segments is reminiscent of results obtained with *Heterodontus* immunoglobulin heavy chain genes (Hinds and Litman, 1986; Kokubu *et al.*, 1988a). A sizeable number of V_L-J_L-C_L clusters (at least 40 can be shown to be unique) are present and these do not appear to be closely linked (>13−15 kb); efforts to establish more extended linkage relationships using cosmid cloning are in progress. While both heavy and light chain genes in *Heterodontus* exhibit cluster-type organization, germline joining of light chain genes, which occurs in ~50% of the heavy chain clusters, could not be detected. Translation of the joined heavy chain genes suggests that they are not overt pseudogenes; however, they may lack critical 5′ and 3′ regulatory sequences or be translocated to chromosomal environments where they are inactive (Kokubu *et al.*, 1988a). The functional significance of germline joined genes is not understood.

Although fewer light chain genomic and cDNA (Shamblott and Litman, 1989) clones have been characterized, they appear to be even more related than are the V_H genes that can be classified in a single family (Kokubu *et al.*, 1988a). One interpretation for the remarkably close relationship between the coding as well as non-coding regions of different genes is that they have evolved recently from a common ancestor. It is equally, if not more probable, that these sequence similarities reflect a process of intense gene correction that is acting over entire gene loci, i.e. sequence homogeneity is being maintained by gene conversion (or some related mechanism of genetic exchange) of individual members of the gene family against a prototype gene or multiple members are being corrected against each other. In this regard, the conservation of extended segments of simple base repeats in the J_L-C_L intervening sequence (Figure 2) may be significant. It is not certain whether segmental elements associated with either heavy or light chain gene families recombine preferentially with immediately adjacent segments or whether they participate in inter-cluster (combinatorial-type) rearrangements. Extensive DNA sequence identity between different gene loci may promote rearrangements of segments that belong to different clusters.

The close linkage of V_L and J_L, <380 nt, is similar to the V_H−D_1, D_1−D_2 and D_2−J_H linkage distances reported previously (Kokubu *et al.*, 1988a). In mammalian antigen receptor systems, variation in the distances between recombining segmental elements has been noted and may be a factor in the preferential joining of gene segments (Blackwell and Alt, 1988; Chou *et al.*, 1987). The similar intersegmental distances observed in *Heterodontus* light and heavy chain gene families may reflect their presumed common evolutionary origin and/or may be selected for in order to retain pairing with the most immediately proximal partner, arguing against the possibility of intercluster rearrangement.

In an earlier report, the closer relationship of the *Heterodontus* light chain to higher vertebrate λ versus κ chains was described, although it was noted that there are a few short sequence segments that are shared uniquely between the *Heterodontus* light chain and κ light chains (Shamblott and Litman, 1989). The organization of the light chain genes in *Heterodontus* more closely resembles that of the heavy chain genes found in this species than immuno-

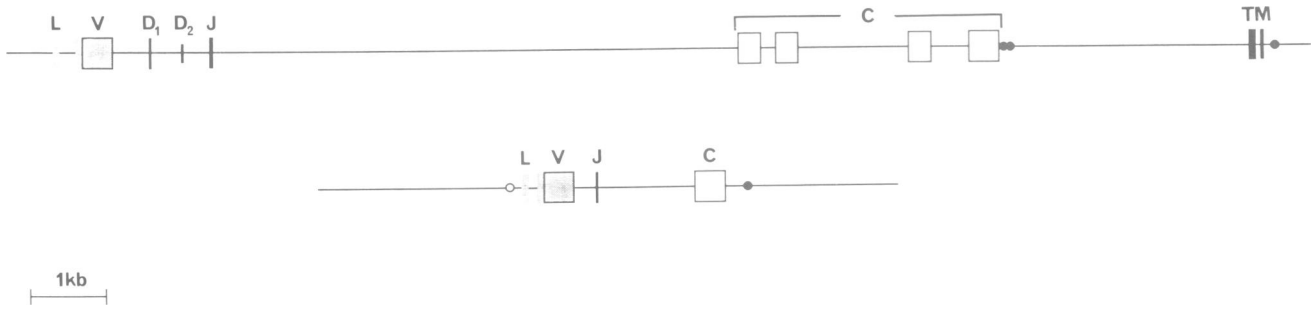

Fig. 3. Representation to scale (shown) of a typical non-rearranged *Heterodontus* immunoglobulin heavy chain gene (top) and light chain gene (bottom). Open shaded rectangles are leader (L) segments, variable (V) segments are shaded and enclosed. D and J segments are indicated by solid bars and constant region exons (C) are shown in open boxes. The transmembrane (TM) exons are indicated by solid bars. Polyadenylation sequences are shown by (——●——); only the light chain gene possesses a regulatory octamer ——— ———.

globulin genes found in any other higher vertebrate. A typical immunoglobulin regulatory octamer, previously shown to be absent in *Heterodontus* immunoglobulin heavy chain genes (Kokubu *et al.*, 1988a), is located upstream of the putative transcription start site in *Heterodontus* light chain genes as well as in the immunoglobulin heavy and light chain genes of all higher vertebrate species characterized to date (Parslow *et al.*, 1984; Litman *et al.*, 1985b; Falkner *et al.*, 1986) including teleosts and amphibians (Amemiya, Haire and G.W.Litman, unpublished data). This sequence appears to be the chief determinant for B cell specificity of both light and heavy chain promoters (Mizushima-Sugano and Roeder, 1986; Wirth *et al.*, 1987). The association of a regulatory octamer with immunoglobulin light chain genes and with both heavy and light chain genes of all more phylogenetically advanced species suggests that in *Heterodontus* the light chain gene system is more modern. The previous interpretation that the heavy chain genes more closely resemble TCRs (Kokubu *et al.*, 1988a), a putatively more primitive form of antigen receptor, is consistent with these observations. Alternatively, the *Heterodontus* heavy chains and TCRs may have lost the immunoglobulin octamer and the light chain actually may be the more primitive form. Tissue-specific expression of *Heterodontus* heavy chain (Litman *et al.*, 1985a) and mammalian TCR (Lee and Davis, 1988) genes presumably is regulated by other sequences. *Heterodontus* may represent the first example where genes encoding different subunits of a single antigen binding heterodimer are regulated through independent mechanisms. The failure to detect close linkage between heavy and light chain gene clusters suggests that these genes are not co-ordinately regulated through a simple *cis* relationship. It is possible that heavy chain gene synthesis is not regulated independently but may be controlled by light chain gene synthesis. It will be of interest to determine whether immunoglobulin-like enhancers occur in the J_H-C_H intervening sequences as have been identified in the J_L-C_L intervening sequences described here.

In *Heterodontus*, heavy and light chain genes are organized similarly (Figure 3); the short distances between the rearranging segmental elements and V-J-C clustering pattern are essentially identical in both systems and contrast markedly with the corresponding iterative patterns of recombining segmental elements and extreme distances that characterize mammalian immunoglobulin heavy (Kodaira *et al.*, 1986; Berman *et al.*, 1988) and κ light chain gene loci (Pohlenz *et al.*, 1987). The organization of the λ locus in mammals (Tonegawa, 1983; Storb *et al.*, 1989) somewhat resembles that associated with primitive vertebrate immunoglobulin genes (Hinds and Litman, 1986; Kokubu *et al.*, 1988a). In an avian, a single functional light chain gene (Reynaud *et al.*, 1985) is the target of extensive gene conversion that utilizes flanking pseudogenes (Reynaud *et al.*, 1987); the heavy chain gene locus is organized and functions similarly; however, multiple D_H segments also can contribute to the generation of diversity in heavy chain genes (Reynaud *et al.*, 1989; J.-C.Weill, personal communication). Three different patterns of gene organization and mechanisms of diversification are associated with the immunoglobulin genes found in species belonging to different phylogenetic classes; however, within a single species, both heavy and light chain genes are organized similarly. It is unlikely that heavy and light chain genes arose independently at different levels of evolution; rather these findings are consistent with the molecular co-evolution of heavy and light chain genes as has been suggested for rDNA (Miesfeld and Arnheim, 1984) and other multigene families (Dover and Flavell, 1984). The coevolution of immunoglobulin gene organization may involve unique, phylogenetic class-specific adaptions in the recombinase system or in other factors that regulate the joining of segmental elements. Coevolution appears to be taking place within an environment in which the organization of segmental elements relates directly to the principal selection event, i.e. the generation of antibody diversity.

Materials and methods

Subcloning

Standard subcloning primarily utilized commercially available M13 and pUC RFs. PCR was employed to estimate the distances between V_L and C_L and to provide DNA sequence overlaps. The design of two oligonucleotides was based on previously described shark light chain cDNA sequences (Shamblott and Litman, 1989): 5′ V_L (5′-CCCAAGCTTGTTCCTGT-CCTCAATCAGA-3′), 3′ C_L (5′-CCCGAGCTCGTCGGAACAAGC GGTGGAG-3′) and incorporated *Hin*dIII and *Sst*I linkers respectively. A 30 cycle PCR reaction, employing reagents obtained from Perkin Elmer/Cetus, used a 94°C denaturation (1 min), 55°C annealing (2 min) and 72°C polymerization (4 min). A final 72°C polymerization (10 min) was used to blunt-end all PCR products. Both intact λ phage and purified λ phage DNA were used as templates. PCR products were digested with appropriate restriction endonucleases and subcloned into M13 for DNA sequencing.

Library construction and screening

The construction, amplification and screening of genomic DNA libraries in λDASH (Stratagene) were as described (Kokubu *et al.*, 1988a). Approximately 1.5×10^6 p.f.u. of the λDASH liver genomic library were screened under moderate stringency hybridization and wash conditions (Litman *et al.*, 1985a). Probe specific activities were $\sim 2.4 \times 10^5$ c.p.m./ml.

Probes

Initially, the *Eco*RI insert of light chain cDNA 1301 was subcloned into pUC18 (Shamblott and Litman, 1989) and the purified insert was labeled by the hexanucleotide random priming method (Feinberg and Vogelstein, 1983). Probes generated by PCR reactions were used in all subsequent library screenings and Southern blot analyses. HFV_L complements 288 nt of the V_L, HFC_L complements 303 nt of the C_L, HFL complements 681 nt of the mature coding region of cDNA 1301 (Shamblott and Litman, 1989) and 122IVS complements ~380 nt of the V_L-J_L IVS of genomic clone λ122. A *Heterodontus* C_H1-specific probe, C_H1_{801} (Kokubu *et al.*, 1987) and a V_H-specific probe, HFV_H (Litman *et al.*, 1985a) also were employed. HSLJ, an oligonucleotide 21mer (5′-CAGGGCGGTCCCAGG-TCCGAA-3′), complementing a relatively conserved portion of the J_L of *Heterodontus* light chain cDNAs 1301 and 1501 (Shamblott and Litman, 1989), was end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase and used as a probe in Southern blot analyses as described (Litman *et al.*, 1985a).

FIGE and Southern blotting

A Pulsewave 760™ controller (Bio Rad Instruments) was employed in FIGE analyses. Twenty microgram blocks of *Heterodontus* erythrocyte DNA were digested with a total of 60 U (two 30 U additions) of *Not*I (New England Biolabs) or *Sst*I (BRL) for 8 h at 37°C. One per cent agarose LE (FMC) gels were cast in 0.5 × running buffer, run parameters were: 165 V for 13.5 h at 14°C, forward polarity intervals ramped linearly from 9 s to 60 s, reverse polarity intervals ramped linearly from 3 s to 20 s. DNA was partially depurinated with a 5 min total UV exposure and denatured in 0.5 N NaOH, 1.5 M NaCl for 40 min. After equilibration, the DNA was transferred overnight by Southern blotting, in fresh 1 M NH_4OAC, 3 M NaCl, 0.3 M Na citrate overnight to Duralon-UV™ (Stratagene). The membrane was UV crosslinked at 1200 μJ employing a UV Stratalinker™ 1800 (Stratagene). Moderate stringency hybridization and washes were carried out as described (Litman *et al.*, 1985a).

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Note added in proof

No evidence has been found for the presence of more than one light chain gene cluster in any of five V_L^+, C_L^+ cosmid (PWE15) clones. (See first paragraph of Discussion.)