Immunoglobulin heavy chain gene organization and complexity in the skate, *Raja erinacea*

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ABSTRACT

Immunoglobulin heavy chain genes from *Raja erinacea* have been isolated by cross hybridization with probes derived from the immunoglobulin genes of *Heterodontus francisci* (horned shark), a representative of a different elasmobranch order. Heavy chain variable (V_H), diversity (D_H) and joining (J_H) segments are linked closely to constant region (C_H) exons, as has been described in another elasmobranch. The nucleotide sequence homology of V_H gene segments within *Raja* and between different elasmobranch species is high, suggesting that members of this phylogenetic subclass may share one V_H family. The organization of immunoglobulin genes segments is diverse; both VD-J and VD-DJ joined genes have been detected in the genome of non-lymphoid cells. J_H segment sequence diversity is high, in contrast to that seen in a related elasmobranch. These data suggest that the clustered V-D-J-C form of immunoglobulin heavy chain organization, including germline joined components, may occur in all subclasses of elasmobranchs. While variation in V_H gene structure is limited, gene organization appears to be diverse.

INTRODUCTION

Immunoglobulin and T cell receptor (TCR) genes rearrange during the somatic development of lymphoid cells belonging respectively to the B and T lineages (1–3). Increasing evidence suggests that the organization of these gene loci is associated with their developmental utilization and expression (2,4). In order to understand the evolution of antibody diversity, we have characterized immunoglobulin gene structure and organization in vertebrate species that occupy critical points in the evolutionary radiations of vertebrates. To date, *Heterodontus francisci* (horned shark) is the most phylogenetically distant species from mammals in which immunoglobulin genes have been characterized. This species exhibits multiple clusters of immunoglobulin segmental elements (V_H , D_H , J_H) which are linked closely to the constant region exons (5). This is in marked contrast to the single, extended array of tandemly linked V_H , D_H and J_H segments that characterize the organization of immunoglobulin heavy chain genes in mammals (1). To determine whether the closely linked form of gene organization is unique to *Heterodontus*,

immunoglobulin heavy chain genes of *Raja erinacea* (little skate), a member of a different order of elasmobranch, have been isolated and sequenced. The genomic organization and structure of immunoglobulin gene segments are shown to be remarkably similar to those found in *Heterodontus*. At least one immunoglobulin gene in *Raja* possesses V_H and D_H segments that are recombined in the germline, and an additional, entirely unique pattern of V_H segment organization is described. These results suggest that the cluster-type of immunoglobulin gene organization extends to include the members of an entire taxonomic subclass and that variations in the arrangement of germline gene segments is extensive.

MATERIALS AND METHODS

Animals

Adult specimens of *Raja* were obtained from the Marine Biological Laboratories, Woods Hole, MA. Tissues were processed immediately after the animals were sacrificed.

Genomic Library

A genomic library was constructed in λ DASH (Stratagene) from *Sau*3A-digested gonad (testes) high molecular weight DNA of an individual specimen of *Raja* as described (6). The library was amplified selectively on *E. coli* P2392 (Stratagene), a P2 lysogen of LE 392. Approximately 2×10^5 recombinant phage representing 0.9 haploid genomes (based on a genome size of 7pg/cell, and an insert range of 17–19kb, assuming proportionate representation) were recovered.

Probes and Library Screening

All probes utilized in the present study were derived from *Heterodontus*. V_H 2809 is a 293 nt *EcoRV*-*ScaI* fragment of a rearranged germline VDJ segment (7). C_H 801 is a germline C_H exon 1 probe containing 81 nt of 5' and 35 nt of 3' noncoding sequence (8). TM/5301 contains 84 nt of C_H exon 4, the transmembrane exons and the 3' untranslated segment of *Heterodontus* cDNA clone 5301 (9). A probe representing approximately one half of exon 1 plus exons 2, 3 and 4 and the secretory exon was derived from *Heterodontus* cDNA clone 6121 (9). DNA probes were labeled by random hexanucleotide priming with the concentration of the N^6 primer adjusted to 10^{-1} that described originally (10). This reduction in primer concentration

results in longer transcripts and facilitates identification of heterologous genes (unpublished observations). Nitrocellulose lifts were hybridized and washed using moderate stringency conditions that were described previously (6).

Genomic Southern Blots

Total high molecular weight DNA was isolated from *Raja* testes (11). Following standard digestion with various restriction endonucleases and electrophoretic separation, the DNA was partially depurinated by UV irradiation, denatured and transferred to Zeta Probe (Bio Rad) in 1M NH_4OAc , $20\times\text{SSC}$ for 20 hours. The filters were baked under vacuum at 80°C for 2 hours.

DNA Sequencing and Sequence Analysis

All subcloning was done in commercially available M13 Rfs and pUC vectors. DNA sequences were determined by the dideoxynucleotide chain termination method (12) using $\alpha\text{-}^{35}\text{SdATP}$ label and modified T7 DNA polymerase, Sequenase (United States Biochemical Co.). The primary strategy used to extend sequences of specific clones or to verify sequences on the opposite strand utilized sequence-specific 18mer extension primers. V_H containing restriction fragments from skate clone Re102 were found to be unstable in both M13 and pUC vectors. For sequencing this clone, twice the recommended concentration of a 50% recombinant/50% non-recombinant mix of pUC12/Re102 V_H XbaI was used and the sequence was primed

internally with an 18-mer extension primer designed for the 5' framework (FR) 1 region of Re107 V_H . The sequence was extended and confirmed on both strands using consequent internal primers. Routine analyses of DNA sequences were made using the IFIND program which is a copyrighted software product of Intelligenetics, Inc., Palo Alto, CA.

RESULTS

Immunoglobulin heavy chain locus genomic organization

Heterodontus V_H (2809) specific and C_H exon 1 (801) probes were used to screen duplicate lifts of a *Raja* gonadal DNA library ($\sim 100,000$ recombinant plaques representing approximately 0.5 haploid genomes). Corresponding plaques that were positive with both V_H and C_H probes were isolated and partial restriction maps of three representative clones are presented in Fig. 1A. The constant region exons were designated 1 through 3 by sequence comparisons to *Heterodontus* constant region exons. The close linkage (10kb) of V_H and C_H elements is reminiscent of the genomic organization described in *Heterodontus*. In addition, seven clones that were positive only with V_H or C_H probes were analyzed. In all cases, the restriction maps are consistent with the V_H or C_H elements being components of a typical elasmobranch immunoglobulin gene cluster. In order to confirm the presence of multiple C_H components, a genomic Southern blot was performed (Fig. 2). *EcoRI*-digested gonadal

Figure 1. Partial restriction maps of representative *Raja erinacea* immunoglobulin heavy chain gene clones. V_H segments are indicated by an open box, D_H segments by a bold black line, J_H segments by an open circle and C_H exons by shaded boxes. C_H exon numbers were assigned by homology with *Heterodontus* gene sequences. A: V_H positive, C_H positive genomic clones. B: V_H positive, C_H negative genomic clone. E = *EcoRI*, S = *SalI*, Ss = *SspI*, X = *XbaI*

DNA from the same *Raja* specimen that provided the DNA for the genomic library was hybridized with *Heterodontus* probes complementing either the complete C_H plus secretory (6121) or transmembrane (TM/5301) exons. The detection of multiple bands hybridizing at coordinate positions with both probes is consistent with a *Heterodontus*-like genomic organization of the constant region. These results are consistent with an overall length of the constant region of at least 4kb.

V_H genomic organization

*Xba*I digestion of genomic clone Re107 yields a 1.5kb fragment consisting of V_H , D_1 , D_2 , J_H segmental elements that are organized similarly to the prototypic forms of *Heterodontus* immunoglobulin genes (Fig. 3). A very high degree of identity between *Heterodontus* and *Raja* immunoglobulin heavy chain genes is evident at the nucleotide sequence level. As in *Heterodontus* (7), the 12/23 spacer rule(s) of recombination signal sequences (RSSs) allow for the inclusion or exclusion of the D_1 segment during joining. *Raja* genomic clone Re110 (V_H^+ , C_H^- , Fig. 1B) contains a 0.9 kb V_H^+ *Xba*I fragment. The complete sequence of this fragment reveals an unusual genomic organization pattern that is consistent with the joining of V_H and D_H segments (Fig. 4). A J_H element in the typical germline configuration is 327 nt downstream of the V_H RSS. The presence of an intervening sequence (IVS) which divides the leader segment indicates that this gene is not processed (see below). This VD-J rearranged clone resembles the germline-

Figure 2. Southern blot of genomic DNA. *Heterodontus* liver (H) and *Raja* testes (R) DNA (10 μ g) was digested with *Eco*RI, electrophoresed, then transferred to Zeta Probe. Duplicate strips from the same blot were probed with 6121 [9] and 5301/TM [9]. Standards are indicated in Kb.

joined V_H genes described previously in *Heterodontus* (7). The heptamer and nonamer sequences located 3' to the joined (V_H - D_H) segment of Re110 are very similar to those found 3' of germline D_2 elements. An intact D_2 segment is not found between the V_H and J_H segments, indicating that the D_2 segment may have been incorporated into the D_H region of Re110 during the joining event. Alternatively, this gene form may represent a pre-segmented V_H region (see below). It is difficult to determine whether the D_1 element has been used or bypassed in this rearranged gene, due to the apparent effects of junctional joining, however, the sequence motif CAACAG is found in both the germline D_1 segment and the D region of Re110 (Figs. 3B and 4).

Clone Re102 reveals a unique pattern of segmental organization. The sequence of a 0.78 kb *Xba*I fragment of this clone is consistent with a VD_1 - D_2 J configuration (Fig. 5). The

Figure 3. Nucleotide and amino acid (one letter code) comparisons of the V_H segments from *Raja* gene Re107 (top line, EMBL accession # X15124) and a prototypic *Heterodontus* clone 1315 (7) (bottom line). A dash indicates predicted amino acid homology. Non-identical amino acids are designated. Recombination signal sequences are highlighted. Re107 V_H gene segment nucleotide sequences are numbered in right margin. Noncoding nucleotides (excluding RSSs) are in lower case. The V_H to D_1 (342 nt), D_1 to D_2 (229 nt) and D_2 to J_H (325 nt) IVSs are not shown. FR and CDR segment boundaries were determined by comparison to previously published *Heterodontus* V_H sequences (7). A: V_H segment. FR1: nt 1 to 90; CDR1: 91 to 105; FR2: 106 to 144; CDR2: 145 to 195; FR3: 196 to 293. B: D_1 segment. Coding unit = nt 29 to 41. C: D_2 segment. Coding unit = nt 29 to 36. D: J_H segment. Coding unit = nt 40 to 87. The J_H region 5' boundary is designated immediately adjacent to the RSS.

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                                (splice) leader      FR1
caatatattttatcctcgccattcatgtctttttttgaacttgcaggtgtccattccgagat 60
ccaggatataatccctcagggtttttgatattttta-----caggtgtccattcagatg
                                D V
V L T C O P K T G G T A V P P G S I T L T C
CGTTCCTCACTCAGCCAGAGACAGGACCGGCTCTGGAGGCTCCATCAGCTGACCTG 120
CGTGTGACTCAGCCAGAGGACAGAGACCGGAAACCTGGAGGCTCCCTGAGACTGACCTG
- - - - - E A E - - G K - - - - - L R - - -
K T S G F T L S CDR1 FR2 V R O S P G
TAAACACAGCGGTTCACTCTTAGCAGCTACTATATATTTGGTCCGTCAGGTCCCGG 180
TAAACATAGTGATTCACTCTTGGCAACACCGGATGTACTGATACGACAGGTTCCTGG
- - - - - N - - G N S R M - - W I - - -
O G L E W L L T Y H A S A T N Y F A P G
GCAAGGCTGGAGTGGCTGCTTACTTACACGCTTCTGCCATAACTACTTCGCTCCAGG 240
GCAGGGCTGGAGTGGTGGTCTTACCTACGCAATAGTATTCGCCCCAGA
- - - - - V H - - D - - S S - - S V - - E
I E S FR3 V R F T P S T D N S N N M F T V I A
GATAGAGAGCCGATTACTCCGTCACAGACAATTCGAATAATATGTTCACCGTGATCG 300
GATTAAGGATCGATTACTGCGTCCAAAGACACTTCAAATAATATCTTCGCTTGGCCAT
- L D - - - - A - K - T - - - - I - A L A M
R N L K I E D T A V Y Y C A D T D O O Q
CAGAACTGAAGATCGAAGACACCGCTGTCTACTGTGCG-----ACAGATCAACAG 356
GAAGAACTGAAGACCGAAGGACCGCATCTATTACTGTGCAAGAAACATTACTACATT
K - - - - T - G - C - I - - - - R N I T T L
G A D
GGGAGATGACACAGCCgttataccaatACAAAAACtccacaacccataataactgtga
GGGTAC---ACTGAGCgttactgtcaatACAAAAACtccctcagcgc-taattgaagag
G
tctggccagtatatgatattttcaatttaatttcatacaacaggagcataaacatgt
tctgggtcagtgcttgc-tttacaatccaactatatacagtggaatttcatttgtgt
cccggaatattttc---cgacatttatgtagaggttaacttgagtttaatttatatttata
ccagcaatgctcactcagcaggtttat-tatggataaaactgatg-aattatcattcatc
tgtagatgattcttatcacatttcactgacctgtt-----taatacacgggg
agttgtgtgatt---tcaataattgctgatattgtgtgagtgaaatataaaacatcagg
ccgtggtatgtgattattgttggaaatgatttttttaatgcattcattgttttcaaagag
ataatgtaccaacag-----tgaaaagaatca--ttagcgcagttagaatttacaatttt
caataaggaattgtttagatgtgaaataacgaataattacaggatttttgttcttctt
-aactctgggatgtg-----gatgtttaa--aataatg--aggccttattctcca--t
tcgcctgcctgtgtgcagatcaTGTATGTgtgaggaagcccatagaatgtaaCACCGTG 760
tc-----tgtcagagaccAGTTATGTgtgacctgcccagggaatgtaaCACITG
J
ACTCTCTTAATACTGGGAGGAGGACGATGGTGACGGTAACCTGCG 808
ACACCTGGACTACTGGGACAGAGGACCATGGTGACGGTGACTGCAG
T M D - - - - - T - - - - - A

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Figure 4. Comparisons of the V_H segments from *Raja* clone Re110 (top line, EMBL accession # X15125) and *Heterodontus* clone 1320 (7) (bottom line). A portion of the leader and leader IVS are shown for the purpose of orientation. Sequence alignment includes the introduction of 19 gaps. Amino acid sequence (in the one letter code) is shown above Re110 and below where different in 1320. In 1320, a dash is used to indicate predicted amino acid homology. Recombination signal sequences are highlighted. Re110 V_H gene segment nucleotide sequences have been numbered in right margin. Non coding nucleotides (excluding RSSs) are indicated by lower case. FR and CDR segment boundaries were determined by comparison to previously published *Heterodontus* V_H sequences (7). V_H segment: FR1: nt 56 to 145; CDR1: 146 to 160; FR2: 161 to 199; CDR2: 200 to 250; FR3: 251 to 343. D segment(s): 344 to 366. J_H segment: 761 to 808. The J_H region 5' boundary is designated immediately adjacent to the RSS.

predicted amino acid sequence of Re102 V_H includes a complementarity determining region (CDR2) which is one amino acid longer than most other elasmobranch V_H regions. The RSSs found immediately 3' of the VD_1 segment are identical to those found 3' of the D_1 segment of the prototypic $V-D_1-D_2-J$ gene Re107, and a 22 base pair spacer is present. The 8 nucleotides inserted between the end of the V segment and the heptamer exhibit a similar nucleotide sequence to non-rearranged D_1 segments. Two hundred forty seven nucleotides 3' to the VD_1 segment is a 5' D_2 -like RSS separated from a consensus J_H by 7 nucleotides that are not related to previously observed D_2 segments (Fig 5). It appears that the D_2 segment has not been deleted in this clone since i) the sequences of the nonamer and heptamer 5' to the J_H element as well as the 12 bp (vs. 22 bp) are characteristic of D_2 and ii) the seven nucleotides found

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                                FR1
A  ID V V L T O P K T G T A V P G G S 50
  GATGTCGTTCTCACTCAGCCCAAGACAGGACCGGGTCTCTGGAGGCTC
  GATGTCGTTGACTCAGCCAGGCGGACAGAGACCGGAAACCTGGAGGCTC
                                E A E G K -
                                CDR1
I T L T C K T S G F T L S VS Y Y M Y FR2 I
CATCAGCTGACCTGTAACACAGCGGGTTCCTTAGCAGCTACTATATGTATTGAT 110
CCTGAGACTCACCTGTAAACTAGTGGATTCAATCTTGGCAACACCGGATGTACTGGAT
L R - - - - - N - - G N T R - - W -
                                CDR2
R O V P G O G L E W L A Y H A P T T T
CCGTGAGTCCCGGGGAGGGCTGGAGTGGCTGCTTACCACGGCCGACTACCA 170
ACGACAGGTTCCTCCGGGACGGGCTGGAGTGGTGGTTCCTACTACGATGCTTCATCCAGCAA
- - - - - V H - - D - - S S S N
                                FR3
V V F A P G I E S F T P S K D N S N N
CGTCTACTCGCTCAGGGATCGAGAGCCGATTACTCCGTCGAAGACACCGATGTATATTACTGTC 230
TAGTTAC---GCCCCAGAGATTAGGATCGATTACTGCTCCAAAGACACTTCAAATAA
S - - - - E I K D - - - - A - - T - -
M F T V I A R N L K I E D T D V Y Y C A
TATGTTCCCGTTCAGGATCGAGAGCCGATTACTCCGTCGAAGACACCGATGTATATTACTGTC 290
TATCTTCGCTTGGCCATGAAGAACCTGAAGACCGAAGGACCGCATCTATTACTGTG
I - A L A M K - - - - T - G - I -
                                D
R D V G
GAGA--CATGTGGGTACACGGTctgcaaacagcgacttctcctgtACAAAAACtgggaca 358
AAGAAACATTACTACATTGGGTACACTGAGCAGttactgtcaatACAAAAAC
- N I T T L G T
ccaagtttaactcctgtgtcaacctacaagcagctagagagccgtgtgaagcaaatcaa
atgaatttcacccgttaaaagtacatggattgttttgagaatgtccaaactgtcttcggac
caattaacgtttattttacatgagcttttagaagaacagcgtttacgtttacaagtgacagcg
aagagtcctcagggacatcatctctgttagctgtacatgcatca
                                L * Y Y P D
gttcttgaagagcgAGTATTGAcattgcatgtttCACTGTCTATAGTACTATCCTGAC 647
AGTTATTGtgaccctgcccagggaatgtaaCACITGTGACCATGGAC
T M -
                                J
Y W G G G T M V T V A C S G 684
TACTGGGGAGGAGGAACCATGGTGACGGTAACCTGCG
TACTGGGACAAGGGACCATGGTGACGGTGACTGCAG
Q - - - - A

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Figure 5. Nucleotide and amino acid (one letter code) comparisons of the V_H segments from *Raja* gene Re102 (top line, EMBL accession # X16146) and *Heterodontus* clone 1320 (bottom line). Nucleotide sequence alignment includes 2 gaps. Amino acid sequence is designated above Re102 and below where different in 1320 (7). Recombination signal sequences are highlighted. Non-coding nucleotides are in lower case. The putative D_1 to D_2 IVS is indicated, but unaligned as it is not present in *Heterodontus* clone 1320. FR1: nt 1 to 90; CDR1: 91 to 105; FR2: 106 to 144; CDR2: 145 to 198; FR3: 195 to 294; putative D_1 region: 295 to 304; J_H segment: 639 to 684; putative contribution of D_2 : 630 to 638. FR and CDR segment boundaries were determined by comparison to previously published *Heterodontus* V_H sequences (7). The J_H segment 5' boundary was determined by comparison to germline *Raja* J_H segments.

5' to the start of the consensus J_H sequence are consistent with a DJ joining event. The putative D_2 segment in this clone possesses a stop codon which is in frame with respect to the J_H coding region.

DISCUSSION

Skates and rays diverged from sharks during the Triassic period, approximately 220 million years ago. Since both shark and the skate have similar, clustered immunoglobulin heavy chain genomic organizations, it is reasonable to assume that all elasmobranchs may exhibit the same pattern. Some time prior to the divergence of sharks and skates, the immunoglobulin genes in a putative ancestor underwent unit duplication, resulting in the genomic organization described here. The functional significance of this gene organization pattern and the mechanism(s) of divergence of the mammalian organization pattern are of considerable interest and biological significance.

Joined germline V_H segments also have been conserved in evolution since the shark-skate divergence. Due to the high frequency of these genes in gonadal DNA libraries, the presence of intact IVSs and the fidelity of their joins, it is unlikely that

Shark1320J _H	TMDYWGQGTMTVTA
	: : : : : : : : : :
Re102J _H	YPDYWGCGTMTVTS
	: : : : : : : : : :
Re107J _H	YLDYWGE GTMTVTS
	: : : : : : : : : :
Re110J _H	SPNYWGCGSMTVTC

Figure 6. Predicted amino acid comparison of *Raja* and *Heterodontus* J_H regions. Germline J_H regions from *Raja* clones Re102, 107 and 110 are aligned. The top sequence represents the J_H from the prototypic shark clone 1320 (7).

these genes represent processed pseudogenes or incompletely rearranged germline genes. Furthermore, most of these genes are potentially functional as they possess known regulatory elements and lack termination codons within the coding regions. The unique VD₁-D₂J configuration found in Re102 represents the third variation of germline fusion events, the first two being VD-J and VDJ forms (7). The VD₁-D₂J gene presented here has a stop codon in the putative D₂ segment. While this may imply that 102V_H is a pseudogene, the stop codon may be obliterated by N-region diversity during D₁-D₂ joining. Both *Heterodontus* and *Raja* exhibit the VD-J arrangement; the VDJ configuration has not been detected in the skate, although 3/13 non-preferentially selected *Heterodontus* clones are in this configuration. The VD₁-D₂J form is prevalent in the skate. Of the 9 genomic clones examined, 4 are VD₁-D₂J, 1 is VD-J joined and 4 are unrearranged. It is unknown if the other VD₁-D₂J genes possess the in frame stop codon and the extra CDR 2 amino acid. It has been suggested that germline joined genes may reflect the stabilization of at least a portion of antibody specificities in the germline (7). In this way, chance somatic recombination may be partially avoided for certain segmental gene pairings that are necessary in host defense. Other independent gene clusters (non-joined) would be available for typical rearrangement. The presence of similarly joined genes in skate indicates that these germline joinings may have taken place prior to the evolutionary divergence of shark and skates. Alternatively, the joined genes may reflect the structure of ancestral immunoglobulin genes prior to the complete segmentation of the genes through inter- or intragenic recombination or by insertion of mobile recombination elements as has been suggested (13–15). The high degree of nucleotide relatedness between V_H segments of genes found in all forms suggest that fusion of segments (or invasion by IVSs) occurred in one or a few V_H precursors prior to extensive duplication. Had the different V_H genomic organizations arisen from an individual precursor(s), the level of similarity between all V_H genes probably would have been lower, however, extensive gene conversion may serve to stabilize this unique multigene family.

The clustered immunoglobulin heavy chain gene organization of elasmobranchs would seem to preclude ordered, sequential expression of multiple constant region isotypes. The Rajiformes, however, express two antigenically distinct molecular weight classes of immunoglobulin heavy chains (16,17). In *Bathyraja aleutica* (Aleutian skate), these two classes of immunoglobulin are co-expressed on individual embryonic spleen cells. Adult spleen cells express only one or the other class (18). Thus, the possibility that a developmentally controlled, sequential expression of multiple immunoglobulin heavy chain isotypes may occur in this species is currently under investigation.

Skate IgH V segments exhibit a very high degree of nucleotide homology, on the order of 90%, including CDR1 and CDR2 (data not shown). This is reflected in a high level of amino acid homology. While it seems possible that several overlapping genomic clones have been isolated (Fig. 1), this degree of homology is seen between all V segments examined to date, including rearranged genes and has been noted previously with *Heterodontus* V_H genes. Shark and skate immunoglobulin heavy chain V_H genes also are closely related. For the examples shown, FRs of the skate genes are approximately 78% identical to equivalent portions of the shark genes. Predicted amino acid sequences indicate an overall homology of 60%. The most highly conserved segment of the V_H region is FR2 (amino acids 36 to 48) which exhibits an amino acid homology of approximately 80%. It is likely that elasmobranchs share an immunoglobulin V_H family. In these species, variation that ordinarily is associated with gene subsets may not be present or may be compensated through variability in gene organization and presumably recombination mechanisms (7). The process whereby widely disparate V region gene segments are produced in higher vertebrates may likely be a recent adaptation and may be associated with the evolutionary shift from the cluster organization to the tandemly linked segmental element (mammalian-type) pattern of immunoglobulin gene organization. Nucleotide and amino acid identities are much lower in comparisons of complementarity determining regions (CDRs), as would be expected. J region nucleotide identity is about 70% for the examples shown. Within the skate, the predicted amino acid sequences of J_H regions ranges from 60% similarity for Re110 and Re107 to 87% for Re102 and Re107. This is in marked contrast to the shark, where only a single amino acid differs in the 3' portions of eight different *Heterodontus* J_H genes, representing three different forms of segmental organization (7) (Fig. 6). The D₁ segments of the non-rearranged genes, Re107 and shark 1315 are nearly identical. The D₂ segments differ in length, but exhibit similar nucleotide motifs; D₁ and D₂ segments presumably are present in all V-D-J-C clusters. The similarity of D segments between sharks and skates is consistent with a greater role for joining imperfections and N additions in the diversification of antibody V regions, rather than for D-coding differences. In both sharks and skates, RSSs are highly conserved and are characteristic of the segments they flank which probably is important in the selective utilization of immunoglobulin gene segments.

IVSs exhibit a low degree of nucleotide homology between skates and sharks, ranging from 15% identity with the incorporation of 9 gaps in the alignment for the D₁-D₂ IVSs of Re107 and shark 1315 (not shown), to 49% homology with two gaps for the D₂-J IVSs of Re110 and shark 1320 (Fig. 4). The D₂-J IVS is consistently the most conserved within the V coding unit. The high level of nucleotide homology of this IVS is retained across a number of shark/skate comparisons (data not shown). The significance of this finding is unclear but suggests that these genetic regions may be important functionally and not serve as spacers alone. The low degree of homology of IVSs contrasts with the high level of sequence identity found in V_H gene segments, implying that these genes do not represent a recent duplication event and that selective pressure to retain V elements does not operate at the level of the entire V-C linked cluster.

Skate genomic immunoglobulin heavy chain clones do not possess the invariant regulatory octamer found upstream of the TATA box in higher vertebrates. The octamer also is lacking in *Heterodontus* immunoglobulin heavy chain genes.

In summary, the structure and organization of immunoglobulin gene segments found in representative elasmobranchs belonging to different phylogenetic orders are remarkably similar. These findings suggest that this unusual type of gene organization may extend throughout the class *Chondrichthyes* and may have changed only with the emergence of the *Osteichthyes* (bony fishes) (Amemiya and Litman, unpublished). The relative adaptive advantages of the different forms of gene organization are of considerable significance and understanding these may provide unique insight into the relationships between the linkage of rearranging gene segments and their functional utilization.

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