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Evolution

V_H gene organization in a relict species, the coelacanth *Latimeria chalumnae*: Evolutionary implications

(segmental elements/diversity segment/genomic library/molecular phylogeny)

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ABSTRACT **The living coelacanth *Latimeria chalumnae* is a relict species whose higher-level phylogenetic relationships have not been resolved clearly by traditional systematic approaches. Previous studies show that major differences in immunoglobulin gene structure and organization typify different phylogenetic lineages. To date, mammalian-, avian-, and elasmobranch-type gene organizations have been identified in representatives of these different phylads. A fourth form or organization is found in *Latimeria*, which possesses immunoglobulin heavy-chain variable region (V_H) elements separated by ≈190 nucleotides from diversity (D) elements. Adjacency of V_H and D elements is characteristic of the elasmobranch "clustered" arrangement, although many other features of coelacanth V_H gene organization and structure are more similar to those of bony fishes and tetrapods. These observations strongly support a phylogenetic hypothesis in which *Latimeria* occupies a sister-group relationship with teleosts and tetrapods.**

Resolution of the structure and organization of immunoglobulin genes has provided valuable insight into genetic mechanisms that diversify the immune response. Owing to the similarities between the rearranging genes that specify the immunoglobulin and T-cell antigen heterodimers, it has been postulated that the segmental rearrangement mechanism emerged early in vertebrate evolution. During the past several years, our laboratory has sought to characterize the origins of this mechanism and to identify alternative mechanisms that may be used to diversify germ-line genetic material. In the course of these studies, we have demonstrated that representative elasmobranchs (sharks and skates) possess an immunoglobulin heavy-chain (IgH) gene organization that differs markedly from that of mammals (1–4). In the elasmobranch system, the individual structural components [heavy-chain variable region (V_H), diversity (D), joining region (J_H), constant region (C_H)] and accompanying recombination signal sequences (RSSs) (5) are analogous to their mammalian counterparts. However, elasmobranch IgH germ-line genes are arranged in discrete "clusters" (i.e., V–D_1–D_2–J–C), an organization highly reminiscent of the genes for mammalian δ and γ T-cell antigen receptors (2). The distances between the rearranging segmental elements in both heavy and light chains (6) are consistently <350 nt, a feature that appears to be unique to this lineage. Furthermore, this linkage arrangement is characteristic of a second isotype gene identified in the skate (7) as well as in a representative of an independent lineage of cartilaginous fish, a holocephalan (J.P.R., C.T.A., and G.W.L., unpublished observations). In contrast, ray-finned (bony) fishes and anuran amphibians all appear to possess a mammalian-type IgH gene arrangement (8–10), indicating that the clustered organization is probably restricted to the cartilaginous fishes. In this paper, we describe features of the V_H gene organization of the living coelacanth, *Latimeria chalumnae*, and discuss the ramifications of these observations in view of a recent suggestion that actinistians (coelacanths) may be most closely related to the cartilaginous fishes (11, 12).§

MATERIALS AND METHODS

Isolation of *Latimeria* DNA and Construction of a Genomic DNA Library. *Latimeria* muscle tissue (≈10 g) was obtained from a female specimen (catalog no. 08118) archived at the Virginia Institute of Marine Sciences and DNA was isolated by a standard procedure (13). A total of ≈200 μg of DNA was isolated. Gel electrophoresis in a 0.4% agarose gel indicated that the mean molecular size was >50 kb. A genomic bank was constructed from *Sau*3A partially digested DNA in Lambda DASH II (Stratagene) and a total of 2.9×10^5 primary recombinants were obtained; this represents an ≈1.5 times coverage of the *Latimeria* genome, assuming a mean insert size of ≈18 kb, a haploid genome size of 3.6 pg (14), and unbiased clone representation.

Genomic Library Screening. An amplified portion of the library ($\approx 3 \times 10^5$ total clones) was screened by using a heterologous (shark) V_H probe, HfV_H (1), labeled via N^6 random priming (15) to a specific activity of 2×10^9 cpm·μg^{-1}. Filters were hybridized in 0.6 M NaCl/0.02 M EDTA/0.2 M Tris·HCl, pH 8.0/0.5% SDS/0.1% $Na_4P_2O_7$ (SET) for 12 h at 65°C using 5×10^5 cpm of labeled probe per ml. Filters were washed at 52°C using four 15-min changes of 1× SSC (1× SSC = 150 mM NaCl/15 mM sodium citrate)/0.1% SDS/0.05% $Na_4P_2O_7$ and autoradiographed for 3 days at −80°C using a single intensifying screen. A number of positive-hybridizing phage clones were identified and four were plaque-purified and characterized extensively.

Subcloning and Sequencing. Selected V_H^+-hybridizing restriction fragments were subcloned into M13mp18/19 (Pharmacia) or Bluescript SKII+ (Stratagene) vectors and sequenced on both strands by the dideoxynucleotide chain-termination method (16) with Sequenase (United States Biochemical). Alignments were made via GENALIGN and IFIND (IntelliGenetics); where appropriate, gaps were manually introduced to maximize similarity.

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Abbreviations: V_H, variable region of heavy chain; IgH, immunoglobulin heavy chain; D_H, diversity region of heavy chain; J_H, joining region of heavy chain; C_H, constant region of heavy chain; RSS, recombination signal sequence; FR, framework region; CDR, complementarity-determining region.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. X57353, X57354, X57355, and X57356 for 15011b, 15011a, 15021a, and 15022, respectively).

Genomic Southern Blotting. Southern blot hybridization of *Latimeria* DNA was carried out with a homologous V_H probe. Coelacanth muscle DNA (7.5 μg) was digested with *Eco*RI or *Sst* I, electrophoresed in a 0.8% agarose gel, and transferred to a Sureblot membrane (Oncor, Gaithersburg, MD) following the manufacturer's recommendations. A *Latimeria* V_H probe prepared from the coding region of 15021a (see Figs. 1 and 2) was labeled via N^6 random priming to a specific activity of 1.5×10^9 cpm·μg^{-1}. The blot was hybridized in SET for 12 h at 72°C using 2×10^6 cpm of labeled probe per ml. The blot was washed at 65°C using three 15-min changes of 1× SSC/0.1% SDS/0.05% $Na_4P_2O_7$ and then at 72°C using three 15-min changes of 0.5× SSC/0.1% SDS and autoradiographed.

PCR Amplification of V_H. *Latimeria* genomic DNA was subjected to PCR amplification using a ubiquitous V_H framework 1 (FR1)-specific primer containing a 2-nt terminal spacer and 5′ *Eco*RI site (CCGAATTCTGWCCTGYAMAGCCTCYGGNTT) and a ubiquitous V_H FR3-specific primer containing a 2-nt terminal spacer and 5′ *Bam*HI site (CCGGATCCGCACAGTAATAVRYRGCNGTGTCYTC) (M is A or C; N is A, C, G, or T; R is A or G; V is A, C, or G; W is A or T; Y is C or T). Amplification conditions were as follows: denaturation, 94°C for 45 sec; annealing, 55°C for 30 sec; extension, 72°C for 1 min for 35 cycles with 1 μg of original DNA template. The PCR product was digested with *Eco*RI and *Bam*HI and subjected to electrophoresis on a 3% agarose gel. The fragment was excised, subcloned into M13mp18/19, and sequenced.

RESULTS

Identification and Restriction Mapping of V_H Genes. The partial restriction maps of the four V_H^+ clones are illustrated in Fig. 1. Three of the clones possess two or more hybridizing components, which were subsequently shown to represent V_H elements. One λ clone possesses a single V_H element. Hybridization of these λ clones with various heterologous J_H- and C_H-specific probes failed to reveal additional hybridizing elements, even though these probes exhibit considerable cross-species hybridization (see below).

V_H Gene Sequences. Eight V_H-containing restriction fragments were subsequently subcloned and sequenced. Four of these are pseudogenes with termination codons in all three reading frames of the coding region; the remaining four contain potentially functional V_H genes. Three of these sequences are illustrated in Fig. 2. *Latimeria* V_H elements possess a 5′ transcriptional regulatory octamer (ATGCAAAG) that differs by 1 nt from the consensus ($T^8 \rightarrow G$) (17), and an A+T-rich region ≈20 nt downstream, the presumed functional equivalent of a TATA sequence. A leader peptide sequence encoding 19 amino acids is split by an ≈140-nt intervening sequence with consensus splice sites. The V_H sequence from FR1 through the end of FR3 spans 294–297 nt (98–99 amino acids), and its RSS V_H heptamer and nonamer sequences are typical and separated by a 23-nt spacer. The inferred amino acid sequences from the four potentially functional *Latimeria* V_H genes are given in Fig. 3. Absolute nucleotide and inferred amino acid identities between the four *Latimeria* V_H coding sequences are 82–86% and 73–83%, respectively, and most of the differences are concentrated in the second complementarity-determining region (CDR2; Figs. 2 and 3). By definition, the four sequences are members of the same V_H family.

Southern Blot Analysis of *Latimeria* V_H Genes. Southern blotting confirms the nature of the extensive V_H gene family in *Latimeria* (Fig. 4). Complex banding of varying intensity is apparent in digests obtained with both restriction enzymes. A significant portion of the hybridizing components may represent pseudogenes as is suggested by DNA sequencing (see above). Furthermore, internal cleavage within V_H may account for some of the band complexity. Conversely, many of the bands may consist of multiple V_H elements.

PCR Amplification of *Latimeria* V_H Sequences. To investigate the possibility that V_H families other than those detected by the *Heterodontus* (shark) probe were present in *Latimeria*, a PCR strategy using degenerate primers to the FR1 and FR3 regions of V_H genes was adopted. A 5′ primer ending at a conserved phenylalanine just upstream of CDR1 and a 3′ primer at the 3′ end of FR3 were devised that should amplify all lower vertebrate V_H genes described to date, with the exception of some of the more divergent *Xenopus* families (10, 18). This primer set gives a band of expected size in ratfish (*Hydrolagus*), shark, gar (*Atractosteus*), sturgeon (*Acipenser*), lungfish (*Protopterus*), as well as *Latimeria*. PCR products have been sequenced from *Heterodontus*, *Protopterus*, and *Latimeria* to confirm their identity as V_H genes. Nucleotide sequence identity lower bounds for those PCR products that were sequenced were 69% within species (lungfish) and 53% among species (*Heterodontus* vs. *Latimeria*). This range could presumably be expanded with the introduction of sequences from other species. In all, 18 such clones from *Latimeria* were sequenced and can be grouped as 14 different V_H genes (data not shown). All of these differ

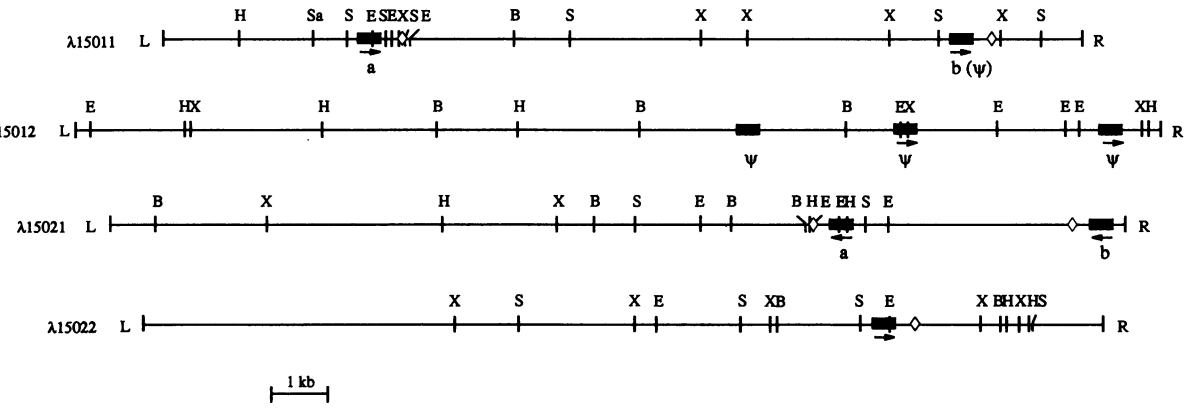

FIG. 1. Restriction maps of four V_H-containing λ clones from a *Latimeria* muscle DNA genomic library. Enzymes used to generate the maps were *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), *Sst* I (S), *Sal* I (Sa), and *Xba* I (X). Left and right arms, respectively, are designated L and R. Boxes denote relative positions of V_H elements; when position is not precisely known, boxes are shown in the center of the restriction fragments to which V_H fragments were localized. Arrows specify transcriptional orientation (5′ → 3′) of the respective V_H elements within individual clones and ψ denotes a V_H element inferred to be a pseudogene (see text). Diamonds indicate locations of apparent D elements (see text and Fig. 2) that were identified via sequence analysis.

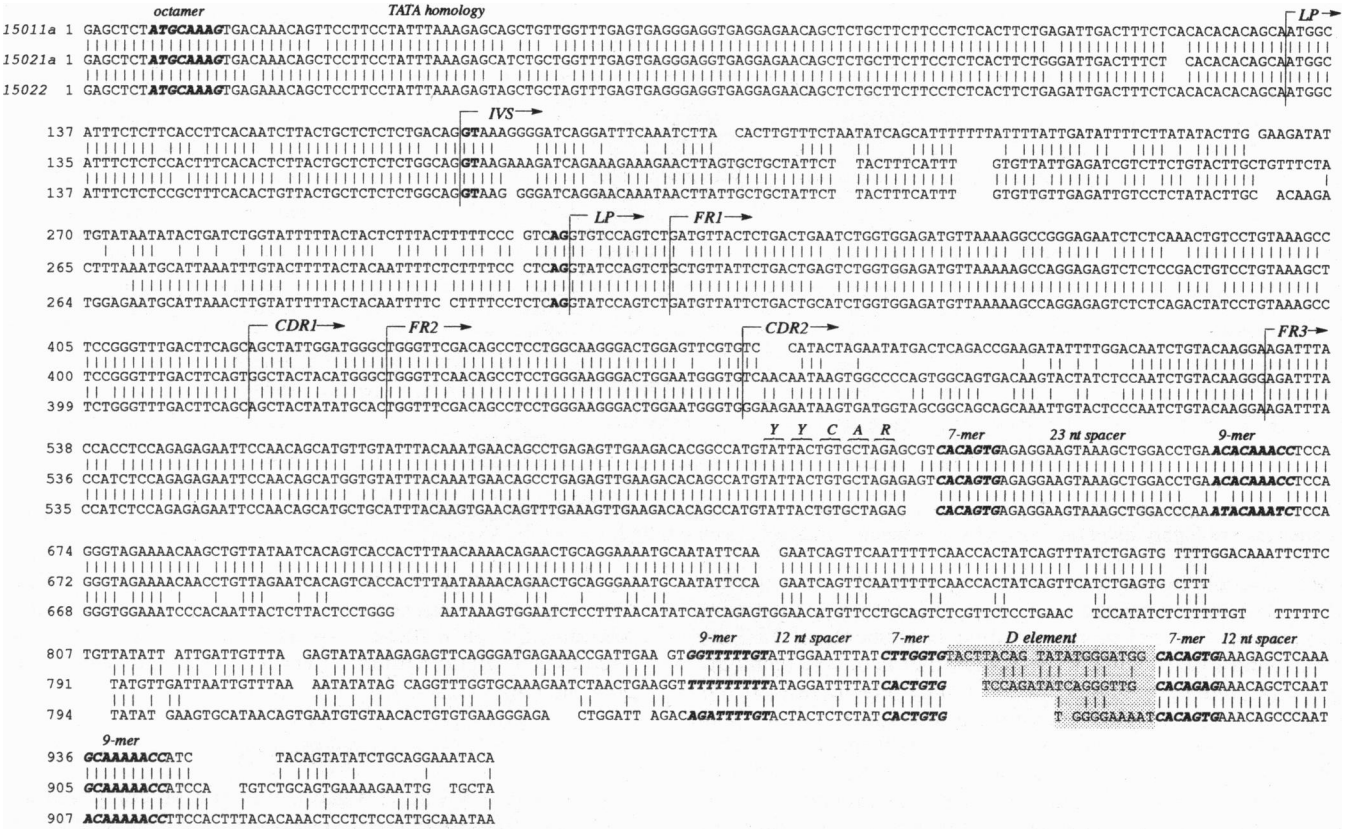

FIG. 2. Alignment of three *Latimeria* genomic V_H sequences. LP and IVS refer to the split hydrophobic leader peptide and intervening sequences, respectively. Consensus splice signals in the IVS are boldface and the V_H octamer and all heptamer/nonamer RSSs (including those associated with the presumptive D elements) are boldface and italicized. Presumptive D elements are indicated by shading. Selected V_H-hybridizing restriction fragments were sequenced and analyzed as described (6–8). A fourth V_H element, 15021b, was also sequenced but is not shown in this alignment because much of its 5′ region was not contained in the λ clone from which it was subcloned (see Fig. 1). 15021b was considered to be a potentially functional V_H gene on the basis of its open reading frame from FR1 to FR3 (see Fig. 3). (The sequence of 15021b is available on request.)

from each other but are similar to 15021a, a *Latimeria* V_H prototype, and show 75–81% overall nucleotide identity, thus indicating that they represent members of the same family. While other V_H families that were not amplified in this experiment may exist in *Latimeria*, these presumably are highly divergent. The most effective means to approach this problem involves the use of an iterative cDNA screening approach (18), which is precluded in this species, since suitable lymphoid tissue is not available.

Identification of *Latimeria* D_H Segments. Extended sequencing downstream of the V_H elements unexpectedly reveals the presence of putative D elements. These elements are flanked by presumptive RSSs with 12-nt spacers and are ≈190 nt distal to the nonamer sequence of the potentially functional V_H elements and one pseudogene (Figs. 1 and 2). Comparison of the putative D elements and associated RSSs of *Latimeria* and those of several vertebrate species is given in Fig. 5. The near-consensus sequences of the heptamers and nonamers and the exact placements of the components [heptamers, nonamers, and spacers (5)] comprising the presumptive RSSs suggest very strongly that these are authentic D elements in the coelacanth. Moreover, despite the short length and notable divergence of D elements in general (which precludes rigorous similarity comparisons), the *Latimeria* D sequences

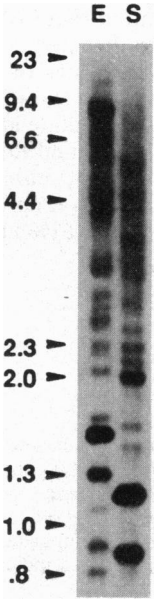

FIG. 4. Southern blot analysis of *Latimeria* genomic DNA digested with *Eco*RI (E) and *Sst* I (S) and subjected to electrophoresis in 0.8% agarose. The blot was hybridized with a homologous V_H probe (15021a) and washed under relatively high stringency conditions as described. Autoradiography was for 12 h at −80°C with one intensifying screen. Sizes are in kb.

	FR1	CDR1	FR2
	1 10 20 30		40
15021a	AVILTESGGDVKKPGESLRLSCKASGFDFS	GYYMG	WVQQPPGKGLEWV
15011a	D-T---------R-----K----------	S-W--	--R--------F-
15022	D----A------------------------	S---H	-FR----------
15021b	D----------R--------------T--	S-E-V	--R--A------I

	CDR2	FR3
	50 60	70 80 90
15021a	STISGPSGSDKYYLQSVQG	RFTISRENSNSMVYLQMNSLRVEDTAMYYCAR
15011a	-ILEYD-DRR -FG-----	---T--------L------------------
15022	GR--DG---S-L-S-----	------------LH--V---K----------
15021b	AY--SR---ST--S-----	------D-----LH------------------

FIG. 3. Alignment of inferred amino acid sequences from four *Latimeria* V_H genes. Dashes denote shared amino acid residues relative to the 15021a prototype sequence.

	9-mer	Spacer	7-mer	D element	7-mer	Spacer	9-mer
Latimeria 15011a	GGTTTTTGT	12	CTTGGTG	TACTTACAGTATATGGGATGG	CACAGTG	12	GCAAAAACC
Latimeria 15021a	TTTTTTTTT	12	CACTGTG	TCCAGATATCAGGGTTG	CACAGAG	12	GCAAAAACC
Latimeria 15021b	GGATTTTAT	12	CACTGTA	TACAAACAGATATTGTGTTGGGAATGTTTGTACATATACAGCAGTTA	CATAGTG	12	GCAAAAACC
Latimeria 15022	AGATTTTGT	12	CACTGTG	TGGGGAAAAT	CACAGTG	12	ACAAAAACC
Latimeria 15011B (ψ)	AGATTTTGT	12	CACTGTG	TATATACGGGGGTGG	CACAGTG	12	ACAAAAACC
Shark 1315 D1	AGTATTTGT	12	CACTGTG	GGTACAGCAGTGGGT	CACGGTG	22	ACAAAAACC
Shark 1403 D1	AGTATTTGT	13	CACTGTG	GGTACAGCGGTGGGT	CACGGTG	22	ACAAAAACC
Shark 2807 D1	AGTATTTGT	12	CACTGTG	GGTACAGCAGTGGGT	CACGGTG	22	ACAAAAACC
Shark 1113 D1	AGTATTTGT	12	CACAGTG	GGTACTACAGTGGGTAT	CACAGTG	12	TCAAATACT
Shark 1315 D2	AGTATTTGA	12	CACTGTG	ATATACTGGGTGG	CACAGCA	12	ACAAAAACT
Shark 1403 D2	AGTATTTGA	12	CACTGTG	ATATACTGGATGG	CACAGCA	12	ACAAAAAGT
Shark 2807 D2	GGTATTTGA	12	CACTGTG	ATATACTGGATGG	CACAGCA	12	ACAAAAAGC
Shark 1113 D2	GGTTTTTGT	22	TACCGTG	TTATACTGGGTGG	CACAGCA	12	ACAAAAAGT
Chicken D1	GGATTTTGG	12	CACTGTG	GTTGTAGTGCTTACGGTTGTGGTGCTTAT	CACGGTG	12	ACAAAAACC
Chicken D2	GGATTTTGG	12	CACCGTG	GGTAGTGCTTGTTGTGGTCCTTAT	CACGGTG	12	ACAAAAACC
Chicken D8	GGATTTTGG	12	CACCGTG	GGTAGTGCTTACTGTTGGGATGCTGAT	CACAATG	12	ACAAAAACC
Rabbit D1B	GATTTTGTG	12	CACCGTG	TAGCTACGATGACTATGGTGATTAC	CACAGTG	12	ACAAAAACC
Mouse DQ52	GGTTTTGAC	12	CACAGTG	CAACTGGGAC	CACGGTG	12	ACAAAAACC
Mouse DSP2.2	GATTTTTGT	12	TACTGTG	TCTACTATGATTACGAC	CACAGTG	12	ACAAAAACC
Mouse DFL16.1	GCTTTTTGT	12	TACTGTG	TTTATTACTACGGTAGTAGCTAC	CACAGTG	12	GCAAAAACC
Human DQ52	GGTTTTTGG	13	CACTGTG	CTAACTGGGGA	CACAGTG	12	ACAAAAACC
Human D2	GGATTTTGT	12	CACTGTG	AGGATATTGTAGTGGTGGTAGCTGCTACTCC	CACAGTG	12	CCCAAAGCC
Human D5	GGATTTTGT	12	CACTGTG	AGAATATTGTAATAGTACTACTTTCTATGCC	CACAGTG	12	CCCAAAGCC

FIG. 5. Comparison of presumptive *Latimeria* D elements and associated RSSs with those of other vertebrate species (2, 19–23). Spacer sequences are highly divergent among some species and have been omitted.

coincidentally exhibit notable identity with a shark composite ($D_1 + D_2$) consensus sequence (Fig. 6). Further downstream sequencing (>1 kb) of the putative D elements of 15021a, 15022, and 15011b failed to detect another D or a J_H element, which would be predicted in the typical elasmobranch V_H configuration. Similarly, hybridizations of the four V_H-containing genomic clones with J_H probes from shark, bichir (*Polypterus*), ladyfish (*Elops*), and mouse did not produce detectable signals. In addition, parallel screening of the genomic library with a series of degenerate J_H-specific oligonucleotide probes that complement all known J_H sequences failed to identify any clones that were V_H^+, J_H^+. Although *Latimeria* J_H elements may be too divergent to be identified in this manner, this is unlikely since relaxed hybridization conditions were used and J_H^+ controls representing several taxa all exhibited some degree of cross-hybridization (data not shown). Likewise, hybridizations of the *Latimeria* genomic library and the four V_H-containing clones with shark, ratfish, bichir, and ladyfish C_H (μ-type) probes failed to yield positive signals. The failure to detect C_H^+ genomic clones could be due to low nucleotide homologies of the various C_H probes, small representation of the library, and potential paucity of C_H genes in the *Latimeria* genome. It should be emphasized that recovery of V_H genes from this library, which was derived from a less-than-ideal DNA sample, was facilitated greatly by their apparent high copy number. This cannot be taken as an indication that low or single copy number genes should have been detected.

Comparison of V_H Sequences. Similarity searches of the GenBank data base using FASTDB (IntelliGenetics) show that the *Latimeria* V_H sequences exhibit highest nucleotide identities (65–70%) with V_H genes of several higher and lower vertebrate species, including mouse, rabbit, caiman (*Caiman*), *Xenopus*, and ladyfish; the shark HfV_H (1) probe used to isolate the *Latimeria* V_H genes exhibits ≤61% identity. It is important to note that there are numerous difficulties in using V_H sequences *per se* for inferring phylogenetic relationships since these exist as multigene families where unequivocal identification of vertically related pairs is not possible (24).

```
                    AG G A          A
Shark composite   TAC TACAGTTATACTGGG TGG
      15011a      ---T----- ---- ----A---
      15021a        -C------- -A---T--
      15011b (ψ)          T------G---G---
      15021b      ---A--------- ...----A...
      15022                       ----GAAAAT
```

FIG. 6. Alignment of presumptive *Latimeria* D elements with a composite D sequence ($D_1 + D_2$) from *Heterodontus* (2). Shaded region encompasses a section of the consensus D_1 sequence; open-boxed region encompasses a section of the consensus D_2 sequence. Dashes denote shared nucleotides with the shark composite sequence. Gaps were introduced to maximize similarity; ... indicates omission of nucleotides from the alignment (cf. Fig. 5).

DISCUSSION

The *Latimeria* V_H gene organization can be compared and contrasted to those of other vertebrates. Like elasmobranchs (but not bony fishes, anuran amphibians, or mammals), *Latimeria* possesses adjacent V_H and D elements. Elasmobranchs, however, possess two D elements with 12/22 and 12/12 (or 12/12 and 22/12) nt spacers, respectively, whereas *Latimeria* has only one D with 12/12 nt spacers (Fig. 2), which would be expected to recombine with a J_H segment possessing a 23-nt spacer. Also, unlike the elasmobranchs, individual *Latimeria* V_H segments do not appear to be linked closely to J_H and C_H components based on extended sequencing, heterologous hybridization, and physical distance considerations relative to V_H, J_H, and C_H coding regions in other vertebrates (1, 5, 9, 19). As with bony fishes, anuran amphibians, and mammals (but not elasmobranchs), *Latimeria* possesses a V_H transcriptional octamer, close proximity of different V_H elements, and presumably large numbers of pseudogenes. While genomic D elements have not been definitively mapped in bony fishes or anuran amphibians, these vertebrate groups do not show the close V–D organization observed in *Latimeria* (8–10).

Systematic studies of the actinistia have met with considerable equivocation (11, 12, 25, 26), a situation not unlike that of the giant panda (27). Proposed phylogenetic relationships of *Latimeria* relative to the selected taxa include *Latimeria* as sister group to the elasmobranchs (12), *Latimeria* as sister group to tetrapods (26), and *Latimeria* as sister group to teleosts and tetrapods (25). While the panda's relationships were clarified via a molecular systematics approach, molecular-level analyses on *Latimeria* have yielded conflicting results (28–34). The 28S ribosomal RNA-encoding DNA (rDNA) sequences support a sister-group relationship between *Latimeria* and tetrapods (28), whereas 18S rDNA sequences generate an ambiguous hypothesis (29). Amino acid sequence comparisons of β-hemoglobins suggest a close coelacanth–tetrapod relationship; however, this conclusion does not hold true for the corresponding α-globin chains (30). Moreover, exhaustive reexamination of the data from the globin study (31, 32, 34) fails to draw the same conclusions. In contrast, mitochondrial DNA sequences (33) suggest that

lungfish (which we have not yet examined extensively) are more closely related to tetrapods than is *Latimeria*.

Although strict sequence comparisons of V_H elements are unreliable as metrics of phylogenetic relatedness (24, 35), IgH gene organization has been shown to differ substantially among major phylads (4). Elasmobranchs possess a T-cell antigen receptor-like clustered arrangement of IgH genes (1–3), whereas teleosts, anuran amphibians, and mammals all appear to possess an extended locus, reiterative-element organization (4, 8–10). A third, highly divergent, IgH organization evolved within the avian lineage (36) and consists of a single functional V_H gene that undergoes gene conversion (with upstream V_H pseudogenes) to generate antibody diversity. Notably, reptilians possess V_H gene configurations that are entirely consistent with those observed in contemporary mammals (37), although the extended V_H gene family is found at more than one chromosomal location (unpublished observations). The data from *Latimeria* genomic V_H sequences demonstrate a V_H gene organization somewhat different from any previously described but are consistent with current views on the early evolution of immunoglobulin genes (1, 4, 5, 8, 38) and may be phylogenetically informative. This is based on our contention that intersegmental distances in immunoglobulin genes are phylotypic with respect to the independent derivation of the cartilaginous and bony fish lines and that their phylogenetic application is removed from the selective exclusions that often need to be made with systematic inferences generated from sequence data alone. The use of this parameter also is of significance since "form" (in this case, genomic length) affects function and frequency of proximal recombination (5).

In using immunoglobulin gene organization for systematic inference, it is important to note that by outgroup comparison, the cyclostomes (jawless fishes—i.e., hagfish and lamprey) do not possess the inducible, heat-stable immunoglobulins seen in the gnathostomes (jawed vertebrates) (38–42). Moreover, recent molecular investigations cast much doubt as to whether the weak, inducible humoral immune response in cyclostomes is at all genetically related to the immunoglobulin gene system in gnathostomes (40–42). Hence, phylogenetically, the genes encoding stable, heterodimeric immunoglobulin molecules (as first seen in chondrichthyans) are shared-derived (synapomorphic) characters uniting all gnathostomes. The proximity of *Latimeria* V_H and D elements is viewed as a vestige of the elasmobranch "clusters" and interpreted as a shared-primitive (symplesiomorphic) character. Presence of the immunoglobulin V_H transcriptional octamer and close linkage of V_H elements are common to *Latimeria*, bony fishes, and tetrapods and would represent synapomorphic characters. The absence of the D element immediately downstream of each V_H element is seen in (and may be synapomorphic for) bony fishes and tetrapods. *In toto*, this interpretation is most consistent with the argument that *Latimeria* occupies a sister-group relationship with the bony (ray-finned) fishes plus tetrapods, a hypothesis receiving some support on morphological grounds (25), but not well-favored in general (11, 26). Furthermore, numerous morphological and immunological synapomorphies are known to define the tetrapods [e.g., possession of lymphopoietic bone marrow (38)] and, when taken into consideration, lead to the suggestion that *Latimeria* may occupy an intermediate position between the cartilaginous and bony fishes. The findings from our study and the lack of congruence, to date, of other molecular systematics studies, somewhat parallel the unresolved quandary in *Latimeria* based on morphological studies. Additional experiments to empirically test our hypothesis, including examination of "primitive" bony fishes and a lungfish, are needed.

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