Volume 13 Number 19 1985 Nucleic Acids Research

Dynamic gene interactions in the evolution of rabbit V_H genes: a four codon duplication and block homologies provide evidence for intergenic exchange

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Received 7 June 1985; Revised and Accepted 5 September 1985

ABSTRACT

Two rabbit V_Ha-negative genes, RVH831 and RVH832, were isolated from a single genomic fragment selected by hybridization with the mouse V_HIII gene S107V1. RVH831 is a pseudogene with a frameshift mutation in FR3 and a 19 bp deletion within the V_H-D splice site. In contrast, RVH832 has an open reading frame and an intact V_H-D splice site and thus may be functional. However, RVH832 displays a unique 4 codon duplication/insertion in FR1 that may be the result of an unequal exchange event between two ancestral V_H genes. Sequence comparisons between these and other rabbit V_H genes reveal patterns of shared blocks of nucleotide substitutions, suggestive of gene conversion. A high overall homology ($\geq$73%) between the compared V_H nucleotide sequences suggests that rabbit V_H genes may not be organized in clearly divergent families or subgroups.

INTRODUCTION

The heavy chain variable region (V_H) of rabbit immunoglobulin (Ig) molecules has been the subject of extensive serological and biochemical characterization since the first description of V_H allotypes by Oudin (1). Four serologically defined V_H subgroups (V_Ha, V_Hw, V_Hx, and V_Hy) have been described (2-4). Each subgroup displays a set of allelic products (allotypes) that appear to be inherited in a simple Mendelian fashion. The genes encoding members of each subgroup are closely linked and segregate as four distinct V_H haplotypes (5). It has been suggested that allotypes present in the V_H regions of rabbit Ig molecules may have conferred a selective advantage during evolution by serving as targets for regulation in an allotype/idiotype network (6-9).

The structural basis for the expression of serologically detectable V_Ha allotypes has recently been reviewed by Mage *et*

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al. (10). A comparison of protein sequence data derived from amino acid sequencing and the sequencing of cDNA clones indicates that differences at 15 amino acid positions clustered within the first and third framework regions (FR1 and FR3) correlate with the expression of the V_Ha allotypes. Thus, the rabbit V_H allotypes represent complex alleles, differing from each other by 5 to 17% in amino acid sequence. However, the manner by which these residues might contribute to the serological determinants is unknown. Amino acid sequences have not yet been correlated with the allotypes of the V_Hw, V_Hx, or V_Hy subgroups.

We (11) and others (12,13) have begun to investigate the organization and evolution of rabbit V_H genes by cloning and sequencing genomic V_H fragments. In this report the nucleotide sequences of two closely linked rabbit V_H genes are compared to other rabbit genomic and cDNA clones. A variety of segmental homologies and, in one V_H gene, an unusual four codon duplication/insertion in FR1, suggest that extensive sequence exchanges and unequal recombination have contributed to the evolution of rabbit V_H genes.

MATERIALS AND METHODS

Isolation and characterization of rabbit V_H clones

The cloned mouse V_H cDNA, pS107V1 (generously provided by L. Hood and S. Crews) (14), was used as a probe to select genomic fragments from a Charon 4A rabbit sperm DNA library (generously provided by T. Maniatis) (15). The S107 probe was radiolabeled with ^{32}P by nick translation with *E. coli* DNA polymerase I (Worthington). Plaque hybridizations were performed at 68°C in 6X SSC (16). Each phage isolate was repurified 4X and retested for homogeneity.

Phage DNA was prepared by polyethylene glycol precipitation of cleared plates (17) and digested with restriction enzymes. Fragments bearing V_H hybridizing regions were identified by Southern transfer and rehybridization with the S107 probe (18). Positive fragments were subcloned into the EcoRI or PstI site of the plasmid vector pUC9 (19). Subclones were identified by insertional inactivation of the *lacZ* region, restriction mapping, and rehybridization to S107.

DNA sequencing

DNA fragments were prepared by electrophoresis, followed by chromatography over NACS-52 resin and end-labeling with ^{32}P using T4 polynucleotide kinase (BRL). Labeled fragments were digested, electrophoresed, and autoradiographed to yield detailed restriction maps. Nucleotide sequencing was performed by a modification of the method of Maxam and Gilbert (20). An additional T+G reaction ($KMnO_4$) was also performed (21).

RESULTS

Rabbit V_H clone isolation

Comparisons between the amino acid sequences of previously reported rabbit and mouse V_H regions suggested that V_Ha-negative genes would hybridize with genes of the mouse V_H subgroup III (22,23). Based on this analysis, a cDNA plasmid clone of S107 (mouse V_H subgroup III) was used as a probe to identify and select hybridizing clones from a rabbit genomic library. Five different phage isolates (of an original 40) survived four rounds of plaque purification and rehybridization with S107. Two DNA segments bearing rabbit V_H genes were subclones from the strongest-hybridizing phage isolate (lambda 8-3) which contained a 10.7 kb genomic fragment. Clone RVH831 is a 1.6 kb EcoRI fragment, and clone RVH832 is a 1.2 kb PstI fragment. Both clones have been mapped and sequenced (Figs. 1 and 2).

Comparison of the nucleotide sequences of RVH831 and RVH832 with the mouse S107 gene reveals overall homologies within the coding regions of 66% and 63%, respectively. However, when subregions are compared, the rabbit V_H genes share higher homology with S107 in the framework regions (67-83%), with low homology in the complementarity-determining regions (CDR) (22-47%) (not shown).

RVH831 is a pseudogene

RVH831 contains several substitutions that result in the loss of conserved amino acid residues (at positions 11, 32, 41, 48, and 68). As shown in Fig. 2, the gene also shows critical deletions, including a single bp deletion (G) at codon 71 that results in a reading frame shift. This shift leads to the loss of several highly conserved residues from the 3′ end of the

A) RVH831

EcoRI DdeI HincII PstI HpaII AvaII PstI EcoRI

B) RVH832

PstI NcoI DdeI BamHI DdeI TaqI HpaII AvaII PstI

100bp

Figure 1. Restriction maps and sequencing strategy for rabbit germline genes A) RVH831 and B) RVH832. Bars indicate coding regions.

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                                        Leader                                   *
     Met Glu Thr Gly Leu Arg Trp Leu Leu Leu Val Ala Val Leu Lys
831  ATG GAG ACT GGG CTG CGC TGG CTT CTG CTG GTC GCT GTG CTC AAA GGT AAT GAT GGA GAA CGC GGG GCA CTG AGT CTG GGA GAG
832  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .

                                                                                      *        L   *1
                                                                                      Gly Val Gln Cys Gln Glu Gln
831  GAC GTG AGT GAG AGA CCC AAA /AG TGT GAG TGA CAG CTT CCT GAC CAA GAC GTC TGA GTT TGC AGGT GTC CAG TGT CAG GAG CAG
832  ... ... ... ... ... .A. .G. G.. ... ... ... ... TG. AT. ... ../ ... A.. ..T ... ... ..A. ... ... ... ... /// ...
                                                                                      Asp  .   .   .   .   /   .

             FR1             10                                                20
     Leu Val Glu Ser Arg Gly Gly                 His Val Thr Pro Arg Glu Ser Leu Lys Leu Phe Cys Lys Ala Ser Gly Phe
831  CTG GTG GAG TCC CGG GGT GGC /// /// /// /// CAT GTC ACG CCT AGG GAA TCT CTG AAA CTC TTC TGC AAA GCC TCT GGA TTC
832  ... AA. C.. ... G.A ..A ..A GCC GGA GGA GGC .TA ... .A. ... ... .G. ..C ..A G.. ... ... ... ... ... ... ... ...
      .  Lys Gln  .  Gly  .   .  Ala Gly Gly Gly Leu  .  Lys  .   .  Gly  .   .  Glu  .   .   .   .   .   .   .

             30 *        CDR1        *               40      FR2                                  *50
     Thr Ile Ser Ser Ser Ile Trp Met Cys Trp Val Arg Gln Ala Leu Gly Lys Gly Leu Glu Trp Val Gly Cys Ile Tyr Thr Gly
831  ACC ATC AGT AGC AGC ATC TGG ATG TGC TGG GTC CGC CAG GCT CTA GGG AAG GGG CTG GAG TGG GTC GGA TGC ATT TAT ACT GGT
832  T.. C.. ... ... ... TA. ... ..A ... ... .G. ... ... ... .C. ... ... ... ... .T. ... A.. ... ... ... ... G.. T..
     Ser Leu  .   .   .  Tyr  .  Ile  .   .  Gly  .   .   .  Pro  .   .   .   .  Val  .  Ile  .   .   .   .  Ala Cys

             CDR2            60              *                   70                  FR3                 80
     Gly Arg Ser Thr Tyr Tyr Ala Gly Trp Val Asn Gly Arg Phe Ser Ile Ser Arg Glu Lys Thr Gln Asn Thr Val Ser Leu Gln
831  GGT CGT AGC ACA TAC TAC GCG GGC TGG GTG AAT GGC CGA TTC TCC ATC TCC AGA GAG AAA ACC CAG AAC ACG GTG TCT CTG CAA
832  A.. G.. ... ..T ... ... ... A.. ... ... ... ... ... ... A.. C.. ... ... ..C .TC GA. ... .G. ..A .GT .GC ..A ...
     Ser Gly  .   .   .   .   .  Ser  .   .   .   .   .   .  Thr Leu  .   .  Asp Ile Asp  .  Ser  .  Gly Cys  .   .

                                                  90
                                 Thr Arg Pro Cys Ile Thr Val Gln Asp
     Met Asn Ser Leu Arg Ala Ala Asp Thr Thr Leu Tyr Tyr Cys Ala Arg   7-mer      21 bp spacer      9-mer
831  ATG AAC AGT CTG AGA GCT GCG /AC ACG ACC CTG TAT TAC TGT GCA AGA CA///////////////////GCTGAGCCTAGATAGAAACCTC
832  C.. ... ... ... .C. ..C ... G.. ... G.. A.. ... ... ... ..G ... G.CACAGTGAGGGGCCCTAGG..GC./......C.C....A.A
     Leu  .   .   .  Tyr  .   .  Asp  .  Ala Met  .   .   .   .   .
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Figure 2. Nucleotide and predicted amino acid sequences of RVH831 and RVH832. Amino acid positions are numbered according to Kabat et al. (22). Dots indicate identity to RVH831. Slashes denote gaps inserted to maximize sequence alignment. Underlined sequences include intron/exon boundaries and V_H-D splice sites.

predicted translational product. A translation of the presumed original reading frame (3′ to the deletion) also contains nonconsensus residues (at positions 88 and 91), indicating substitutions in this region as well. The most significant defect is a large deletion of 19 bp at the 3′ end of RVH831, which results in the loss of the heptamer and much of the 23 bp spacer segment of the V_H-D splice recognition site.

Duplication within RVH832

RVH832 is more likely to be a functional V_H gene in that it has a complete open reading frame and an intact V_H-D splice site. RVH832 does, however, have amino acid substitutions at a few otherwise invariant positions (*e.g.* 32, 37, 69, and 91). RVH832 also has a unique sequence difference at the 3′ intron/exon junction (AG/AT instead of AG/GT) resulting in a replacement of Gly with Asp at position -4 of the leader region.

The most unusual feature of RVH832 is a 12 bp insertion in FR1 which appears to be a four codon duplication of an adjacent sequence. This region of RVH832 was sequenced several times and in both directions to rule out sequencing error. A portion of a sequencing gel including this segment is shown in Fig. 3A. Because of the repetitive nature of the sequence involved, the insertion may be viewed as either an imperfect tandem repeat or an imperfect inverted repeat (Fig. 3B).

Comparison of rabbit V_H sequences

Assignment of a rabbit V_H amino acid sequence to one of the four serologically defined V_H subgroups can only be accomplished with certainty if the sequence is homologous to one of the V_Ha allotypes. Mage *et al*. (10) have compared 26 protein sequences representing the three allotypes a1, a2, and a3, and have shown that residues at 15 positions are correlated with the V_Ha allotype. In contrast, there are as yet no amino acid sequences correlated with any of the three serologically defined V_Ha-negative subgroups (V_Hw, V_Hx, and V_Hy). As a consequence, sequences not clearly homologous with a1, a2, or a3 are presumed to represent one of the minor V_H subgroups and are designated V_Ha-negative. Comparison of 11 partial or complete V_Ha-negative sequences reveals six amino acid positions (2, 3, 64, 75, 81, and 82A) at which most share residues unique from the V_Ha allotypes

B

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GAACAGTCCGGAGGAGGAGCCGGAGGAGGCCTAGTC
----------›    ‹----------
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Figure 3. A) Portion of a sequencing gel spanning the 12 bp duplication in RVH832. Bases of the sequenced strand (S) and the complementary coding strand (C) are indicated. B) This region may be viewed as an imperfect direct repeat or inverted repeat, as indicated by the arrows.

(Fig. 4). RVH831 and RVH832 encode six and five of these V_Ha-negative-associated residues, respectively.

The nucleotide sequences of nine rabbit germline and cDNA sequences are compared in Fig. 5. Two sequences, CLPRV14 and RVH831, are noteworthy in that they show a high degree of overall homology (94%), sharing 11 otherwise unique base substitutions. They also share several pseudogenic defects, which include the frameshift mutation in FR3 and the 19 bp deletion in the V_H-D

Amino Acid Position	2	3	5	8	10	12	13	16	17	64	65	67	70	71	74	75	81	82A	84	85
VHa-neg	GLU	GLN	*	Gly	Gly	Val	*	Gly	Ser	ASN	Gly	Phe	Ser	*	*	GLN	GLN	ASN	Ala	Ala
RVH831	Glu	Gln	Val	Arg	Gly	Val	Thr	Glu	Ser	Asn	Gly	Phe	Ser	Arg	Thr	Gln	Gln	Asn	Ala	Ala
RVH832	/	Gln	Lys	Gly	Gly	Val	Lys	Gly	Ser	Asn	Gly	Phe	Ser	Arg	Asp	Gln	Gln	Asn	Ala	Ala
VHa1	/	Ser	Glu	Gly	ARG	Val	THR	*	*	Lys	Gly	Phe	Ser	Lys	Thr	/	Lys	Thr	THR	GLU
VHa2	/	Ser	LYS	GLU	Gly	PHE	Lys	ASP	THR	Lys	SER	SER	THR	ARG	ASN	GLU	Lys	*	Ala	*
VHa3	/	Ser	Glu	Gly	ASP	Val	Lys	Ala	Ser	Lys	Gly	Phe	Ser	Lys	Thr	/	(?)	Thr	Ala	Ala

Figure 4. Amino acids found at allotype- and subgroup-associated positions in FR1 and FR3 of rabbit V_H regions (10,12,13,22). Asterisks indicate positions without predominant residues. Capitalized amino acids include V_Ha-negative shared residues and V_Ha allotype-specific residues. Slashes indicate gaps. Boxes enclose the six residues characteristic of the V_Ha-negative sequences.

splice site. This striking similarity between two genes derived from the same genomic library suggests that they are recently divergent allelic or duplicated pseudogenes.

A comparison of the nucleotide substitutions for each of nine rabbit germline and cDNA sequences reveals that about half (49%) are unique to only one of the V_H gene sequences. Of the remaining substitutions (i.e. the 51% shared between two or more V_H genes), many (44%) occur between pairs of genes that bear an obvious relationship. These include very closely linked genes that were isolated from a single genomic fragment (e.g. RVH831 & RVH832, CLPRV10 & CLPRV14, and CL42.VH25 & CL42.VH34, abbreviated as VH25 & VH34) and the related gene pair RVH831 & CLPRV14 discussed above. Other substitutions are shared by pairs or sets of genes related by subgroup assignment (e.g. CLpμ3 & CLpγB1 for V_Ha and CLPRV10 & VH25 for V_Ha-negative).

Of greater interest is the pattern of shared substitutions among the V_H genes that shifts through the length of their sequences. For example, in the intron, CLPRV10 shares a substitution with CLPRV14 at nucleotide position 57, two substitutions with VH25 at positions 61 and 86, another with CLPRV14 at position 112, and a deletion at position 132 that is shared with RVH832 (Fig. 5).

More than half of the shared substitutions (56%) occur in pairs or sets of genes that do not otherwise appear to be closely related, i.e. substitutions are shared by V_Ha and V_Ha-negative

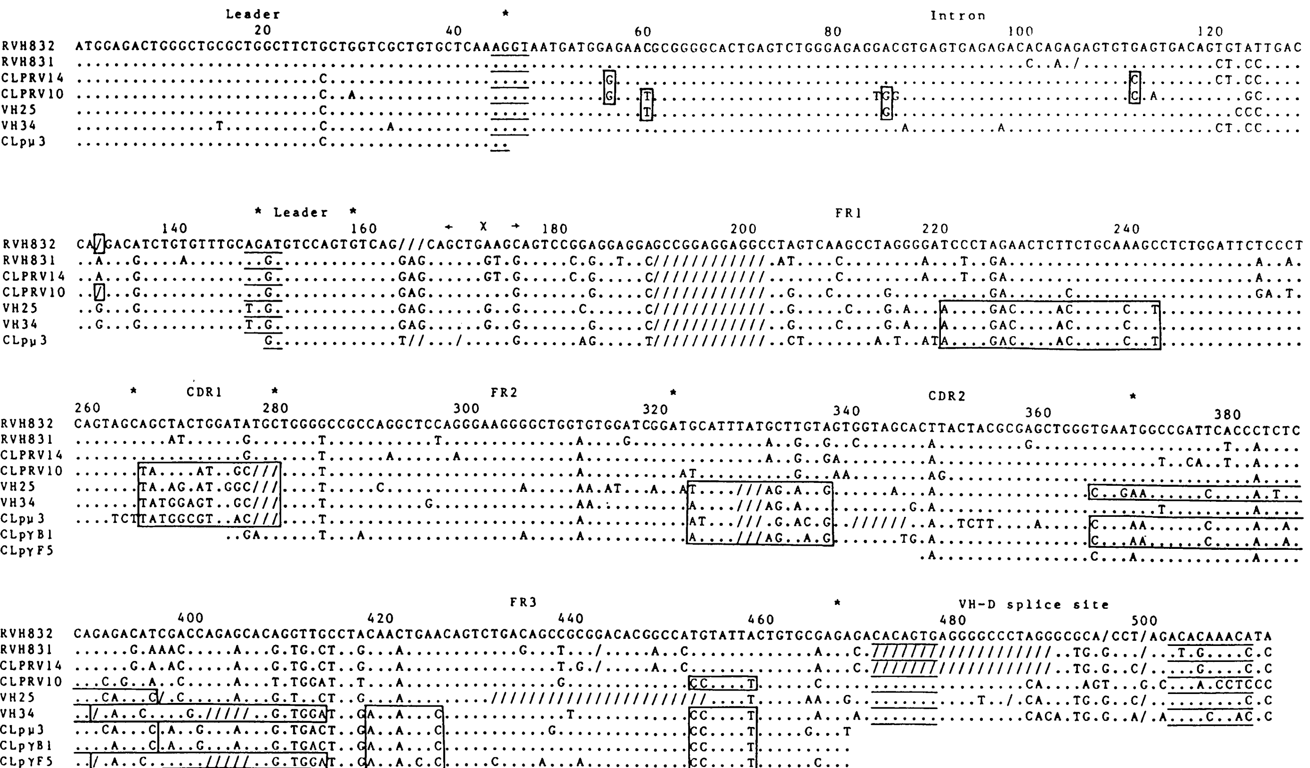

Figure 5. Comparison of rabbit V_H gene nucleotide sequences. Genomic V_Ha-negative genes include RVH832 & RVH831, CLPRV10 & CLPRV14 (12), and VH25 & VH34 (13). CLpγ3 and CLpμB1 are V_Ha2 cDNA clones (24). CLpγF5 is a V_Ha1 cDNA clone (25). Boxes enclose shared substitutions as described in Results. Chi-like sites in FR1 are indicated by χ. Other conventions as in Fig. 2.

genes. For example, CLPRV10 (V_Ha-negative) shares with CLpμ3 (a2) several substitutions in CDR1 and a small block of seven nucleotides in FR3 (Fig. 5). Different patterns of shared substitutions are evident within other V_H genes as well. VH25 and VH34 (V_Ha-negative) each show extensive but different segmental homologies with CLpμ3 (a2) and CLpγF5 (a1). For example, both sequences share several blocks of homology (7-23 bp in length) with CLpμ3 in FR1, CDR1, CDR2, and FR3. However, beginning at nucleotide position 366 and extending through FR3, VH25 and VH34 are significantly different. VH25 most resembles CLpμ3 from position 366 to 399, sharing seven substitutions over this 31 bp segment. From position 400 to the end of FR3, VH25 most resembles the other V_Ha-negative sequences. On the other hand, VH34 resembles a V_Ha-negative sequence from position 366 to 389. From position 390 to 414, VH34 shares with the sequence of CLpγF5 four substitutions and the deletion of six nucleotides. In the remainder of the FR3 sequence of VH34, two additional blocks of seven and eight nucleotides are shared with CLpμ3. These blocks of nucleotide sequence homology represent the genetic basis for the amino acid substitutions previously noted by Bernstein *et al*. (13).

Alignment of the nucleotide sequences of CLpγF5 (a1) and VH34 (V_Ha-negative) was maximized by the introduction of two deletions in the nucleotide sequence, rather than a single deletion of six nucleotides. We have placed a single nucleotide deletion within codon 71 and a 5 bp deletion at codons 75-76. This alignment reveals that a shift in the reading frame of a single DNA sequence, rather than a more extensive series of substitutions, may have led to some of the alternative amino acid sequences observed in this allotypically important region.

Rabbit V_H gene sequence homology

The overall nucleotide sequence homologies for the nine rabbit V_H genes compared in this report (Fig. 6) show that all share $\geq$73% homology, and 30 out of 36 sequence comparisons share $\geq$80% homology. Thus the rabbit V_H genes cannot be subdivided into subgroups or families based on this limited set of DNA sequences. Because two of the three V_Ha sequences used in this

	RVH 832	RVH 832	PRV 14	PRV 10	CL42 VH25	CL42 VH34	CL pμ3	CL pγB1	CL pγF5
RVH832	100	85	86	86	81	82	77	80	78
RVH831		100	94	84	82	81	77	80	74
PRV14			100	86	82	83	77	80	73
PRV10				100	85	86	80	81	84
CL42.VH25					100	87	84	85	81
CL42.VH34						100	84	87	94
CLpμ3							100	91	81
CLpγB1								100	85
CLpγF5									100

Figure 6. The homology between rabbit V_H sequences is given as a percentage of shared bases within the entire V_H coding region, except for CLpγB1 (FR2 through FR3) and CLpγF5 (FR3 only).

analysis are from partial cDNAs that do not include FR1 (24,25), greater divergence cannot be excluded.

DISCUSSION

With the recent cloning of rabbit immunoglobulin cDNAs and genomic fragments encoding V_H genes, a set of nucleotide sequences is now available with which to begin the molecular analysis of the organization and evolution of the rabbit V_H gene complex. Some of the rabbit V_H genes sequenced thus far encode proteins completely homologous with one of the V_Ha allotypes (24,25). Others encode proteins lacking various residues characteristic of the V_Ha allotypes and are thus classified as V_Ha-negative (12,13, and this report). A comparison of the eight nucleotide and three partial amino acid V_Ha-negative sequences reveals six residues unique to this group (i.e. residues not present in a1, a2, or a3 sequences but common to most or all V_Ha-negative sequences) (Fig. 4).

To explain the observation that some individual rabbit V_H genes express characteristics of more than one V_Ha allotype, Bernstein et al. (13) have suggested that the heterogeneity of rabbit V_H sequences may have been caused by the accumulation of mutations in various genes. Alternatively, the composite structure observed among the rabbit V_H genes may be the result of unequal crossing-over (26,27) or gene conversion (28,29). Experimental data suggestive of gene conversion between V genes

have been reported (30-33), and some authors have suggested that pseudogenes may serve as a reservoir of genetic information or even be reintegrated into the pool of functional V genes by gene conversion (28,30). The close proximity of rabbit V_H genes, about 3-6 kb (12,13) compared to 8-25 kb for mouse and human (34,35), might serve to enhance gene conversion.

Comparison of rabbit V_H nucleotide sequences provides several lines of evidence supporting the hypothesis that mechanisms in addition to point mutation have been active in the evolution of rabbit V_H genes. First, the patterns of segmental homology between the examined V_H genes suggest a composite structure for several of the V_Ha-negative genes. The exchange of DNA segments by gene conversion between V_H genes would result in genes that contain nucleotide sequences encoding amino acids characteristic of more than one allotype. A similar sharing of identical subregions has recently been described among human V_κ genes (36), although the shared subregions are smaller in the rabbit V_H genes.

Kenter and Birshtein (37) have noted the presence of sequences in Ig genes resembling the lambda phage Chi site (GCTGGTGG), a promoter of recombination. We have identified Chi-like sites in FR1, CDR1, CDR2, and D regions of rabbit, human, mouse, and caiman Ig heavy chain genes (11). Both RVH831 and CLPRV14 have exact copies of Chi in FR1 (Fig. 5), while the other rabbit sequences differ by 1-3 nucleotides. If these Chi-like sites function as general promoters of recombination in eucaryotes, their presence could facilitate the exchange of V_H segments.

The apparent duplication of twelve nucleotides in FR1 of RVH832 may be the product of such a sequence exchange. For example, an unequal exchange event may have occurred between the RVH832 ancestral gene (V_Ha-negative) and a V_Ha gene, as depicted in Fig. 7A. This model is based on the observation that the overall sequence of RVH832 on the 3′ side of the duplication most closely resembles that of other V_Ha-negative genes, yet 5′ to the duplication RVH832 bears the deletion of codon 2, a feature characteristic of each of the 26 reported V_Ha protein sequences (26). We also suggest that the same genetic mechanism generated

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A.                      1  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17
CLp 3                  CAGTCAGTGAAGGAGTCCGAGGGAGGTCTCTTCAAGCCAATGGATACC
"ancestral" RVH832              CAGGAGCAGCTGGTGGAGGCCGGAGGAGGCCTAGTCAAG
c/o product           CAGTCAG/TGAAGGAGTCCGAGGGAGGTGCCGGAGGAGGCCTAGTCAAG
RVH832                .../...C.....C......GA.....A....................

B.                 95 96 98 98   7-mer       23 bp spacer          9-mer
MVAR10             TTCTGTGCAAGA CACAGTG TTGTAACCACATCCTGAGTGTGT CAGAAATCC
c/o partner                 TTCTGTGGAAGA CACAGTG TTGTAACCACATACCGAGTGTGT CAGAAACCC
c/o = MVAR11       TTCTGTGCAAGA CACGAAGA CACAGTG TTGTAACCACATACCGAGTGTGT CAGAAACCC
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Figure 7. A) A model for the origin of rabbit RVH832 by an unequal exchange between the "ancestral" RVH832 gene and CLpμ3. Bases of the modern RVH832 identical to the hypothetical cross-over product are indicated in the last line by dots. B) A model for the origin of mouse MVAR11 (38) by an unequal exchange between MVAR10 and a hypothetical partner.

an unusual 100 2/3 codon V_H gene of the mouse V_{NP} gene family (38). The extra eight nucleotides in the gene MVAR11 are located at the 3′ end of the V_H coding region and appear to be an imperfect repeat of an adjacent set of nucleotides. This duplication can most easily be explained as the result of an unequal cross-over with recombination points within codon 97 and the V_H-D splice site, as illustrated in Fig. 7B.

These duplications could alternatively be the result of DNA slippage during replication or repair (39-40), a mechanism suggested for the origin of short tandem duplications in the silkmoth chorion multigene family (41). Interestingly, the duplicated sequence of RVH832 bears sequence resemblance to one of the chorion gene repeat units in that both encode multiple glycine residues (41).

In contrast to the mouse V_H gene complex, the organization of the rabbit V_H gene complex is poorly understood. The mouse V_H genes are organized into eight V_H subgroups (families), each of which represents a cluster of closely related genes sharing >80% homology with each other and <70% homology with members of other subgroups (42,43). When the available nucleotide sequences for rabbit V_H genes are similarly compared, all share $\geq$73% homology, with 30 of 36 gene comparisons being $\geq$80% homologous (Fig. 7). Thus, by the criterion used to define mouse subgroups, most of

the rabbit V_H genes (including V_Ha and V_Ha-negative) appear to belong to the same subgroup and none would define a separate subgroup. Alternatively, rabbit V_H subgroups may simply be less divergent than mouse V_H subgroups. This close relationship, if true, could have fostered gene conversion by facilitating interaction between similar V_H sequences. Conversely, extensive gene conversion may have, in fact, served to "homogenize" rabbit V_H genes (27) to the exclusion of clearly defined subgroups. The sequencing of additional rabbit V_H genes, including genes corresponding to each of the serologically defined subgroups, will provide additional insight into the evolution of the rabbit V_H genes.

ACKNOWLEDGEMENTS

The authors thank T. Maniatis for the rabbit genomic library, and L. Hood and S. Crews for the pS107V1 probe. We also thank K.L. Knight and R.G. Mage for sharing sequence data before publication.

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