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Evolutionary aspects of immunoglobulin heavy chain variable region (V_H) gene subgroups

(DNA sequence/saltatory replication/pseudogenes)

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ABSTRACT **We isolated and determined the sequences of two human germ-line heavy chain variable region (V_H) genes and compared them with mouse V_H genes. The results show that the human V_HI subgroup is evolutionarily related to the mouse V_HII subgroup. Evolutionary preservation of homologies in V_H genes of the same subgroup includes not only the coding region but also intron size and homology in noncoding regions. This suggests that a V_H gene subgroup constitutes a multigene family that undergoes concerted evolution. The homology between genes of the same subgroup in different species is greater than that between genes of different subgroups within a species. One of the V_HII genes contains, in complementarity-determining region 2 (CDR2), a 13-base-pair previously shown to be in CDR2 of a V_HIII gene and in a heavy chain diversity region gene, D_H [Wu, T. T. & Kabat, E. A. (1982) *Proc. Natl. Acad. Sci. USA* 79, 5031–5032], suggesting the insertion of diversity region gene sequences into the V_H gene. One of the human V_H genes is a pseudogene because of a terminator, which, together with our previous results, shows that the V_H gene repertoire contains 40% pseudogenes. In one of the V_H genes, direct and inverted repeats at both 5′ and 3′ ends of the gene suggest a potential transposable element that encompasses the entire V_H gene. It is possible that such a structure may facilitate saltatory replication and rapid expansion of V_H gene families.**

The variable region of the immunoglobulin heavy chain is encoded in the germ line in three separate DNA segments: V_H, D_H, and J_H (1–3). Three of the framework regions (FRs) and two of the complementarity-determining regions (CDRs) are included in the coding region of the V_H segment whereas recombination between variable (V), diversity (D), and joining (J) segments is necessary to form the expressed V_H gene. It is likely that there are several hundred V_H gene segments organized in tandem with spacers of 8–15 kilobases (4–6). The multitude of V region amino acid sequences has been subdivided into subgroups that show a higher extent of homology and linkage between some amino acids along the chain (7–9). However, comparison of the amino acid sequences of mouse and human V_H does not always allow the affiliation of V regions from the different species to homologous subgroups (10); the DNA sequences of the V_H genes may provide a better tool for comparison of homologous subgroups in various species. Hybridization with V_H DNA probes showed a correlation between the extent of cross-hybridization and V_H gene subgroups (11). It has been shown that homologous genes of the same subgroup (V_HIII) are physically linked and that genes of different subgroups are not interdispersed and can be ordered on the chromosome by deletion mapping (12, 13). This indicates that throughout evolution the V_H gene subgroup is maintained as a multigene family. A V_H multigene family (subgroup) preserves some characteristics in the noncoding segments that are different from those of other

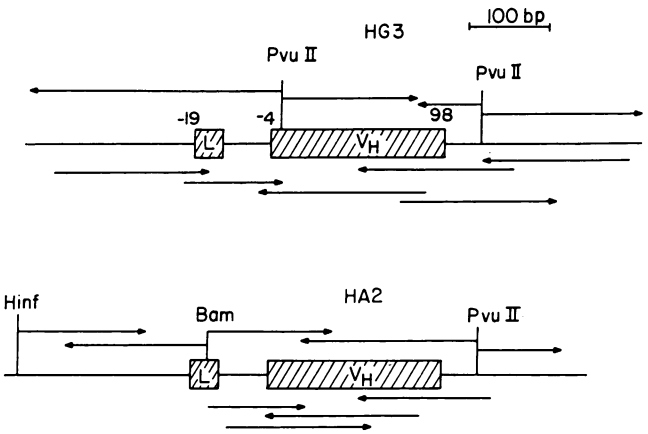

FIG. 1. Strategy for sequence analysis of two human V_H genes. Arrows above the line represent analysis by chemical degradation (19) and arrows below the line represent analysis by M13 cloning (18). ▨, Coding sequences; L, leader.

V_H families (11) and comparison of V_H genes from different species will increase our understanding of the structural features that characterize a subgroup in coding and noncoding regions.

We isolated human germ-line V_H genes by cross-hybridization with mouse V_HII genes (6). The DNA sequence shows that the human genes are homologous to human V_HI (subgroup I), as defined by Kabat *et al.* (14) and we will refer to the human genes analyzed here as human V_HII genes. The homology between mouse and human V_HII genes is preserved in the size [84 base pairs (bp)] and also in the sequence of the intron present at codon 4. Another V_H gene subgroup (V_HIII) contains a longer intron (102 bp) in both mouse and human (11). The homology in both intron and framework codons between mouse and human genes of the same subgroup is greater than between V_H genes of different subgroups in the same species. The sequence also suggests that DNA repeats at the 5′ and 3′ regions flanking the V_H gene can form a large stem-and-loop structure that may facilitate saltatory replication (15).

MATERIALS AND METHODS

A human fetal liver gene library donated by T. Maniatis was screened by plaque hybridization to plasmid pCh104 containing a mouse V_HII gene (6). Restriction endonuclease mapping of purified recombinant phage DNA was carried out as described (11). DNA sequences were determined mainly by cloning and analysis in the M13 mp8 vector (16, 17). Recombinant phage fragments that gave positive hybridization with pCh104 were

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Abbreviations: V_H, D_H, and J_H, heavy chain variable, diversity, and joining regions; CDR, complementarity-determining region; FR, framework segment; bp, base pair(s).

electroeluted from agarose and ethanol precipitated. One or two micrograms of DNA was dissolved in 25 μl of 10 mM Tris·HCl/0.1 mM EDTA, pH 7.4, and sonicated in an Eppendorf tube, using a model W375 sonicator (375 W, 20 Khz; Heat System/Ultrasonics, Plainview, NY). Sonication was done at 4°C by four 5-sec bursts at settings of 4 (output control) and 50% (duty cycle).

The sonicated DNA was subjected to end repair with DNA polymerase I (Klenow fragment) in the presence of 0.1 mM dATP/2 μM [^{32}P]dATP for 30 min at room temperature, followed by phenol extraction and ethanol precipitation. The DNA was dissolved in 5 μl of 10 mM Tris·HCl/1 mM EDTA, pH 7.4, and run on a 1.5% agarose minigel with size markers. The 300- to 500-bp fractions of the sonicated material were collected by cutting a small trough below the 300-bp marker and continuing the electrophoresis for nine consecutive 1-min periods at 20 mA. The nine fractions collected were pooled and ethanol was added. The precipitated DNA was dissolved in 10 μl of 10 mM Tris·HCl/0.1 mM EDTA, pH 7.4, and a portion was ligated to the *Sma* I site of M13 mp8 (10 ng) and used to transform JM101

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HG3   GAAATGGAGC AGGACATGCA TTTCTTCAAG CAGGATTAGG GCTTGGACCA TCAGCATCCC ACTCCTGTGT GGCAGATGGG ACATCTATCT TCTTTCTCAA  100
HA2   -GG---TG-- ---G--CA-- -GC--CT--G  G------TA --GAACC-TT AGCATCCT-- TG-TGG--AA TC--TG--TC -TC---CCA- ---------T
108A                                                                                  TTCCTCTC-A --------C-

                                          I          II
HG3   CCTCGATCAG GCTTTGAGGT ATGAAATAAT CTGTCTCATG AATATGCAAA TAACCTTAGG ATCTACTGAG GTAAATATGG ATACATCTGG GCCCTGAAAG  200
HA2   G--T-        -G-T---- --------GC A-CG------ ---------- ----            ---T A-G-C---A- ---TC-T--T -------G--
108A  TT-A---T - -T-A-T-TA- -CA--G-CCC --- ------ ---------- -T----A--T C-A-GG--TT AA---C-G-- ---TCAACAC -  ------A
                                                                -19
                                                              Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu  293
HG3   CATCATCCAA CAACCACATT CCCTTCTCTA CAGAAGCCTC TGAGAGGAAG ATTCTTCACC ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG
HA2   -----C---- ---------  ----C---AG A--------C- C ----C-CA GC--C----- --- --- --- (         ) TGG A-- CT- --T T--
108A  --A---ATGT -C-A        TG-C----C --C --A-A- ---ACAC-CT GACTC-A--- --- -GA --- -G- --- -TC T-T C-- -T- C-C ---
                  -5
      Ala Val Ala Pro G
HG3   GCT GTA GCA CCA G/GTAAAGGGCC AGCTGGTTCA GGGCTGAGGA AGGGATTTTT TCCAGTTTAG AGGACTGTCA TTCTCTACTG TGTCCTCTCC GCAG/ 390
HA2   -TG -C- --- C-- -/----G----T GC-AAA-C-- -T-AG----- --------GAGG-----CA-- G--G--TC-- -C-A--C--- ----T----T A---/
108A  -CA -G- A-T G-- -/----G----T CA-CAT---CAAAT----A-- --AAAAGAAA-GGCT-G-G-T GTC----A--  --CACT---TCT-T------ T---/

                  1                                  10                                  20
Eu                PCA -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  Ser -  -  -  -  -  -  -  -  -  -
      ly Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
HG3   GT GCC CAC TCC CAG GTG CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG GTT TCC TGC AAG GCA TCT 476
HA2   -C --- --- --- --- --C --A --- --- T-- --- --A --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- --T ---
108A  -C -T- --- --T G-- --C --- --T CA- --- --A --A C-- --- C-- GT- --A --- --- --- --- --- --- A-- --- --- --- --T ---

                     30                                  40                                  50
Eu     -  Gly  -  -  Ser Arg Ser Ala Ile Ile  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  Gly  -  Val  -  Met
      Gly Tyr Thr Phe Asn Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser
HG3   GGA TAC ACC TTC AAC AGC TAC TAT ATG CAC TGG GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA ATA ATC AAC CCT AGT 563
HA2   --- --- --- --- -C- --- --- --- --- A-- --- A-- --C --- --- --- --- --- --C T-C --- --- --- --- TGG --- --- G-- G-C
108A  --- --- --A --- -CT GA- --- A-C --- --- --- --- AAG --- AG- -A- --- AAG A-C --- --- --- --- --- TAT --T T-T --- -A-
                          CDR1                                                                          CDR2

                        60                                  70                                  80
Eu     Phe  -  Pro Pro Asn  -  -  -  -  -  -  -  -  -  -  -  Ile  -  Ala  -  Glu  -  -  Asn  -  Ala  -  -  -  -
      Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr Met Glu Leu
HG3   GGT GGT AGC ACA AGC TAC GCA CAG AAG TTC CAG GGC AGA GTC ACC ATG ACC AGG GAC ACG TCC ACG AGC ACA GTC TAC ATG GAG CTG 650
HA2   AA- --- -A- --- -AG --T T-- --- --- C-- --- --- --- --- --- --- --T --- --- --- --A --T G-- --- --- -C- --- --- C-- ---
108A  AA- --- G-T --T G-- --- AAC --- --- --- A-- A-- -AG -C- --A T-- --T GTA --- -AT --- T-C --- --- -C- --- --- --- --C
                    CDR2
                              90                  98
Eu     -  -  -  -  -  -  -  -  -  Phe  -  Phe  -  -  Gly
      Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
HG3   AGC AGC CTG AGA TCT GAG GAC ACG GCC GTG TAT TAC TGT GCG AGA GA CACAGTG TGAGAAACCA CATCCTCAGA TGTCAGAAACCC TGAGGGAGGA 746
HA2   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -- -----A- ---A--C--- ------G---G------------ C -A------
108A  --- --- --- -C- --- --- --- T-- --A --C --- --- --- --A ---    ------- -T-C----A  ------G--TG------------ -----T -C-

HG3   GTCAGCTGTG CTGAGCTGAG AAAATGGACA GGGGTTATTC AGTTTAAGGA CTGTTTAGAA AACGGGTTAT ATATTTGAGA ACAAAGAACA ATAGAAACAC        846
HA2   -C ------A --- ------ G---------- AA-A------ --A--G-A-- --T-C----- --T-AC---A G-CA--A--   G-------- ---T---TGT
108A    -----TCC T--G-----C --G-GTT-G- -AATAGTCG- TTGCAG-CTT G C------T GCA-TCA--T

HG3   AATCGAATCT AAGAGTTTTA TCCTTCAAGA ATAAGCCAAA CTGACAGAGT GGAAAGGGCC ACACTCAGTA AAGTTGATAC AAACATACCA TAAAAGGTGG        946
HA2   GTA-TTGAGA -TTTAA---- CTTGAG-GT

                                                  II          I
HG3   CTACTATGAA CAAGTTTTTG AATTAGATGA ATAAAAGTCA TTTGGAGGCA AGGTTATTTG GTCATAATGT TAAGAGTAAG CATGATTCCT TACAAAGTGG       1046

HG3   GAAAATTGTC CTTTCAAATG TTTCTGTCAC TTCTTACCCA TAAAGTTCAT TTTAGAGGTT TTAGATTT
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FIG. 2. Comparison of DNA sequences of human and mouse V_H genes. The sequence of *HG3* is translated to amino acids and compared with the V_H amino acid sequence of human protein Eu (14). I and II, the direct and inverted repeats (see text) at nucleotides 136–157 and 1,008–1,016. In the flanking regions, small deletions (empty space) or insertions (above the dashes) were inserted to maximize the homology. Nucleotides identical to those in *HG3* are marked by dashes, CDR regions are underlined, and recombination signals at the end of V_H are boxed. The sequence of mouse gene *108A* (subgroup II) is from ref. 6 and the sequence of human protein Eu (subgroup I) is from ref. 14.

Table 1. Characteristics of V_H gene subgroups

	Comparison	% differences in human and mouse gene segments						Recombination signals, no.
		FR1	FR2	FR3	CDR1	CDR2	Intron	
Human V_HII	*HG3* vs. *HA2*	7	12	6	11	29	32	12
Human V_HIII	*H11* vs. *H16BR*	7	15	13	6	40	19	7
Human V_HII vs. V_HIII	*HG3* vs. *H11*	32	35	23	27	46	>70	37
Human V_HII vs. V_HIII	*HG3* vs. *H16BR*	32	36	23	33	56	>70	39
Mouse V_HII vs. human V_HII	*108A* vs. *HG3*	17	26	21	33	35	53	15
Mouse V_HII vs. human V_HIII	*108A* vs. *H11*	36	48	38	39	56	>70	40
Mouse V_HII vs. V_HIII	*108A* vs. *T15*	37	33	39	33	56	>70	40

Data for *HG3* and *HA2* are from this paper, those for *108A* are from ref. 6, those for *H11* and *H16BR* are from ref. 11, and those for *T15* are from ref. 22.

bacteria. Recombinant M13 mp8 (white plaques) was plated onto a lawn of JM101 bacterial host and, after overnight growth, transferred to nitrocellulose and hybridized to ^{32}P-labeled pCh104. Positive plaques were picked into 1 ml of JM101 and grown for 5 hr, and the DNA was prepared and analyzed as described (18). In some cases, sequence analysis by the chemical degradation procedure (19) was also used.

RESULTS AND DISCUSSION

The Human V_HI Subgroup Is Homologous to the Mouse V_HII Subgroup. On the basis of the amino acid sequences, it is not always easy to relate subgroups from different species, and it was particularly difficult to discern the structural counterpart of the human V_HI subgroup in other species (10). It is shown here and in ref. 12 that the structure of the germ-line genes, including the noncoding regions, allows a better understanding of the evolutionary relationship of the V_H subgroups and thus comparison among V_H subgroups from different species. Several recombinant phages were isolated from a human gene library (20) by cross-hybridization with the mouse V_HII gene-containing pCh104 (6). The sequences of two of these genes (*HG3* and *HA2*) were determined by a combination of the M13 cloning/analysis technique (7) and the chemical degradation procedure (19) as shown in Fig. 1. The sequence of >1,100 bp of a human V_H gene (*HG3*) is shown and compared with that of the human *HA2* gene and the mouse *108A* gene, described in ref. 6, in Fig. 2. The amino acid sequence deduced from the DNA sequence of *HG3* is compared in Fig. 2 with that of a human myeloma protein Eu, belonging to the V_HI subgroup (11). The results show that protein Eu is highly homologous to the predicted amino acid sequence of HG3, differing by only 1 residue in the first 25. This suggests an evolutionary relationship between the mouse V_HII subgroup (*108A* gene) and the human V_HI subgroup. This is further supported by the homology in sequence and size of the intron at codon −4. We have shown previously that mouse V_HII genes contain an intron of 84 bp (6) whereas both mouse and human V_HIII genes contain an intron of 102 bp (12, 21). The results in Fig. 2 show conservation of the intron size between mouse and human V_HII genes and provide further evidence for their evolutionary relationship.

Table 2. Sizes of mouse and human gene segments

	Signal peptide, codons	Intron, bp	V_H, codons
Mouse V_HII	19	84	98
Human V_HII	19	84	98*
Mouse V_HIII	19	103, 159	98, 100*
Human V_HIII	19	103	98*

Data sources are as in Table 1.
* +2 bp between codon 98 and the recombination signals.

Characteristics of V_H Gene Subgroups. To evaluate the homology between V_H genes of various subgroups, we compared the homology of every gene segment within and between subgroups and also between mouse and human genes (Table 1). The results show that, in the framework regions (FR1–FR3), the homology within a subgroup is significantly greater than that between subgroups. Moreover, the homology between mouse and human genes of the same subgroup is greater than that between V_H genes of different subgroups in the same species. The homology in the intron also follows this rule and is even more significant with regard to the size of the intron (Table 2). This analysis indicates strong preservation of noncoding elements, in addition to the coding regions, in V_H genes of the same subgroup.

We have shown previously that V_H genes are organized in clusters of subgroups that are not interdispersed but show physical linkage of the cross-hybridizing genes (12). It is likely that each such cluster of V_H genes is a multigene family that undergoes concerted evolution and preservation of the sequence characteristics of this subgroup (15). The data presented here extend this finding to the V_HII subgroup by comparing the similarity between mouse and human genes.

High Proportion of Pseudogenes. As shown in Fig. 2, codon 6 in *HA2* is a termination codon, TAG. Hence, *HA2* may be a pseudogene that cannot be expressed. It has been shown previously that the mouse V_H gene repertoire is rich in pseudogenes (6, 23). From a larger collection of V_H gene sequences (unpublished data), we can now estimate that 40% of the germ-line genes analyzed (seven mouse and four human V_H genes) drift and become pseudogenes because of terminators or other single-base replacements. This restricts the use of the entire germ-line V_H gene repertoire and shows that there is a conflict between gene diversity and gene expressibility in the immune system. It is possible that mechanisms such as gene conversion or recombination (24, 25) may operate to allow the animal to make use of the coding potential present in these pseudogenes, but this has not yet been shown.

A D_H-Like Sequence in CDR2 of a V_HII Gene. A significant portion of the CDR3 codons in the expressed V_H gene is contributed by D_H gene segments after V–D–J recombination (1–3). This recombination is controlled by highly preserved signals

```
                        50  51  52  53  54  55  56  57  58  59  60
HUMAN VHII(HG3)         ATA ATC AAC CCT AGT GGT GGT AGC ACA AGC TAC

                        50  51  52 52A  53  54  55  56  57  58  59
HUMAN VHIII(VH26)       GCT --T -GT GG- --- --- --- --- --- TAC ---

HUMAN DH(DH2)             - GGA T-T TG- --- --- --- --- TGC TAC -C-
```

FIG. 3. Locations of an identical 13-bp segment in CDR2 of two V_H genes and in D_H2. Numbers above the codons show their positions in the V_H gene as in Fig. 2 (upper line) or as in ref. 26 (middle line). The sequence of D_H2 is from ref. 3.

```
                        10        20
HUMAN   HG3   TCATGAATATGCAAATAACCTT
        HA2   --------------------TGA
MOUSE   108A  ----------------T----A
        108B  ----------------T---GT
        104A  -----------A----T---CA
        111   ----------------C--GCA
        105   ----------------C----G
```

FIG. 4. Sequence of a highly homologous segment in human and mouse genes upstream to the V_H gene. This sequence begins 136 nucleotides 5′ to the initiator ATG and is marked by I and II and by arrows in Fig. 2. Data are from this paper and refs. 6, 24.

located 5′, 3′, and at both ends of the V_H, J_H, D_H, respectively. Hence, the recombination joins the coding segments V–D–J end to end, where D sequences are represented in CDR3. Recently, however, Wu and Kabat (26) showed an identity between a 14-bp sequence present in D_H2 and in the CDR2 of a human germ-line V_HIII gene (V_H26) and suggested a possible insertion of D sequences into CDR2, within the V_H gene. It is of interest that 13 bp of the sequence described by Wu and Kabat (26) are present in CDR2 of gene *HG3* in the same position as in V_H26 (Fig. 3). This 13-bp sequence appears as part of the CDR2 of two different subgroups (V_HII and V_HIII) as well as in the *D* minigene, coding for CDR3. If this identity between CDR2 and D_H2 sequences is not coincidental, it suggests that introduction of D-like sequences into V_H genes, either by insertion (26) or by gene conversion (25), is not limited to only one subgroup and may be an additional factor in generating antibody diversity. An alternative possibility is that the D_H minigenes may have evolved from a segment corresponding to CDR2 of a primordial V_H gene. The significant homology among various D_H genes (3) supports this hypothesis.

A Potential Transposable Element in V_H Genes. Gene sequences conserved between species may be of importance and we have compared noncoding regions of human and mouse V_H genes. In the flanking region 5′ to the V_H gene, we find a sequence of 20 bp (nucleotides 136–157, Fig. 2) that is highly conserved in all mouse and human V_HII genes analyzed (Fig. 4). This sequence begins 124 bp 5′ to the initiator ATG codon and may have a role in the function of the V_H genes. In Fig. 2, this 20-bp long sequence is subdivided into segments marked I and II. It is of interest that in gene *HG3* (Fig. 2) an almost perfect direct repeat of segment I is found in the 3′-flanking region of the V_H gene (nucleotides 1,008–1,016, I in Fig. 2) and is preceded by an inverted repeat of segment II (nucleotides 996–1,007, II in Fig. 2). Hence, segments I and II at both the 5′- and 3′-flanking regions of V_H form a potential stem-and-loop structure that includes the entire V_H gene (Fig. 5). The repeats are reminiscent of the border regions in prokaryotic, viral, and eukaryotic transposable elements (27–29). A similar structure was also found by inspecting mouse V_HII gene sequences published previously (24). The two structures of human and mouse V_H genes depicted in Fig. 5 show remarkable sequence similarity at the stem-and-loop borders, although the sizes of the loops are slightly different. A stem-and-loop structure may function in generating tandemly duplicated sequences leading to amplification of V_H gene families by the saltatory replication mechanism (15). This mechanism suggests that, because of the stem-and-loop structure, several rounds of replication occur at a single initiation site, generating an "onion skin" replicated bubble. After ligation of the ends of the duplicated DNA, these replicated structures may recombine in the chromosome to generate a tandem array of gene sequences vicinal to the original gene duplicated (see ref. 30 for further discussion). Such a mechanism was suggested recently to explain somatic gene amplification and generation of multigene families (29). These events may be very rare, as suggested by the similarity of restriction enzyme patterns of V_H genes in Southern blots of DNA from various human individuals (unpublished data). However, it is possible that, at times of genome instability (perhaps during periods of speciation), saltatory replication may take place. The immunoglobulin V_H gene families can undergo rapid evolutionary expansion (12, 15) and saltatory replication or unequal-crossing over have been suggested to explain this phenomena (15). Saltatory replication is a more attractive mechanism to explain the concerted evolution of immunoglobulin V genes reflected in species-specific residues.

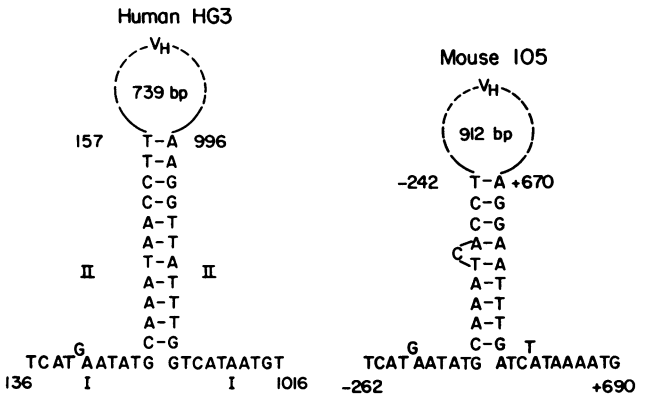

FIG. 5. Schematic representation of the stem-and-loop structure that may be formed between the direct and inverted repeats in V_H genes. (*Left*) Human *HG3* gene. The direct (I) and inverted (II) repeats and the numbers are as shown in Fig. 2. (*Right*) Mouse *105* gene. The sequence and numbers are as given in ref. 24.

Another example for the possible involvement of DNA repeats in transposing DNA segments into genes was recently discussed by Cooke and Baxter (31). They suggested that exon 1 of the prolactin, growth hormone, and chicken conalbumin genes was independently inserted into these genes as a mobile genetic element, as indicated by the direct repeats flanking this region in all three genes.

Regulatory sequences were recently located in immunoglobulin V_H genes within 100 nucleotides 5′ to the V_H coding region (32). Hence, these sequences are included in the V_H gene, which may be replicated in the stem-and-loop structure shown in Fig. 5. The replication of V_H genes by a saltatory replication mechanism may be facilitated by the presence of a transposable element-like structure. This may explain some of the mechanisms operating in generating the large repertoire of V_H genes and processes of rapid expansion of V_H genes during evolution.

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