Proc. Natl. Acad. Sci. USA
Vol. 77, No. 11, pp. 6734–6738, November 1980
Genetics

Evolutionary approach to the question of immunoglobulin heavy chain switching: Evidence from cloned human and mouse genes

(recombination/DNA/antibodies)

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Contributed by Philip Leder, August 7, 1980

ABSTRACT **We have used cloned mouse and human heavy chain genes to identify regions of conserved homology between mouse and man and between μ and α genes. Using heteroduplex mapping, we find that coding segments for homologous domains appear to have been well conserved between the two species, but the short intervening sequences that separate domains have diverged considerably. These studies also identify extensive regions of homology 5′ to both μ and α constant region genes that are conserved between the two genes as well as between mouse and man. These segments encompass regions in which μ/α recombination occurs during the heavy chain class switch, and their extensive homology may be relevant to this process.**

Antibody genes are created by a somatic recombination event that occurs during the maturation of antibody-producing cells (1–5). This process seems to involve a single step for active κ light chain genes: the joining of one of a large number of germ-line variable (V) region genes to one of four joining (J) segments encoded close to the constant region sequence (6–8). This step can generate enormous diversity, not only through the combinational power of joining V and J regions but also because it is variable in its crossover point and thereby creates additional sequence diversity in a critical region of the light chain (6–9).

Recent studies of the heavy (H) chain genes of the mouse suggest that these same processes also operate but that they may involve additional complexities (10–15). It has been suggested that V/J recombination may in some cases require an additional step involving the joining of an additional DNA segment (the D segment) incorporated between V and J (15, 16). Apart from this unique genetic arrangement, H chain expression differs from light chain expression in at least one other important respect: H chain V regions appear to be sequentially (and, in a special instance, simultaneously) associated with different constant regions—first μ, then μ and δ, and finally α or γ or ϵ. Thus, the original V region or antigen-binding specificity is retained by each antibody-producing cell but the effector (H chain) function of its product is changed. This process is referred to as the "H chain class switch."

Cloning of mouse genes has revealed many of the molecular details of this process (10, 11, 13–15). After the μ chain gene has been formed by V/(D)/J recombination, switching to α or γ chain production involves recombination between a region within the intervening sequence of the μ gene and a similarly placed region close to either the γ or the α constant region. Although it is clear that such recombination is accompanied by a deletion of gene sequences between the μ and the newly expressed H chain genes (17–20), its structural basis remains unknown but apparently is different from that of V/J recombination.

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Our studies of human H chain genes have allowed us to take an evolutionary approach to the mechanism of the H chain switch. We have used cloned mouse and human H chain genes to identify regions of conserved homology between mouse and man and between μ and α genes. These studies reveal the conservation of coding sequence homology between the two species and the divergence of the intervening sequences that border each H chain domain. They also reveal an extensive region of homology 5′ to both μ and α constant region genes that is conserved between mouse and man. This conserved segment is situated so as to encompass the μ recombination region and its extensive homology may be relevant to the α switch.

MATERIALS AND METHODS

DNA Cloning and Sequence Determination. The procedures for obtaining and characterizing the germ-line mouse and human μ, γ, and α genes utilized cloned cDNA probes corresponding to mouse μ, γ (21), and α mRNAs. These probes were used to isolate the corresponding genes from the clone libraries prepared from partially size-purified DNA segments or from randomly cleaved, partial restriction endonuclease digests of total mouse 12-day embryo, human placenta, or human fetal liver DNA (22). The procedures used to generate these libraries (22) and to isolate these clones, with the phage λ vectors CH4A (23) and λCH28 (F. Blattner, personal communication), the phage packaging system (24), and an *in situ* hybridization technique (25) have been described.

Electron Microscopic Analysis, *In Situ* Hybridization, and Computer Techniques. Heteroduplex analyses were carried out with twice-$CsCl_2$-banded, intact phage particles destabilized in 200 mM EDTA and then denatured in alkali and neutralized. Hybridization was performed in 50% formamide at room temperature overnight (26). Filter hybridization of restriction endonuclease digests of DNA was performed according to the method of Southern (27). Computer analysis of DNA sequences was performed with the program of Korn and Queen (28) and by the direct dot matrix comparison of Maizel as described (29).

RESULTS

Cloning Mouse and Human μ and α Gene Segments. To develop a set of human and mouse μ and α H chain genes suitable for evolutionary comparison, genomic clones were derived from clone libraries by using DNA isolated from 12-day mouse embryo (J. G. Seidman, personal communication), from human fetal liver (22), and from human placenta (unpublished data). Each clone was characterized by its ability to hybridize to its cloned, mouse-derived cDNA complement and by sequence determinations that identified regions of coding se-

Abbreviations: V, variable; J, joining; H, heavy; kb, kilobase(s).

quence in each relevant clone (data not shown). The size of each cloned segment of DNA, its source, the enzyme used to create the fragment, and its vector are shown in Table 1. Each clone, except MαS107, was isolated in what is presumed to be its germ-line arrangement (nonrecombined). MαS107 was derived from the IgA-producing myeloma S107 and represents a fragment derived from an α chain gene that spans the recombination site used in the μ/α heavy chain switch (see below).

Comparison of Human and Mouse μ Genes. Both human and mouse fragments were cloned in the λCH4A vector and their orientations were determined by restriction endonuclease mapping, R-loop analyses, and partial sequence determination (data not shown). The heteroduplex formed between the 11-kilobase (kb) human segment and the 11.2-kb mouse segment shows two distinct regions of homology (Fig. 1*A*). One is regularly interrupted with homologies corresponding to the four constant μ region coding domains separated by nonhomology bubbles that correspond to the intervening sequences that separate the domains (31, 32). The second homology region is a 1.5-kb-long segment beginning about 1.3 kb 5′ to the first μ coding domain. (Two smaller, discontinuous regions of homology occur 5′ to the major homology segment.) The major noncoding homology segment encompasses the region involved in the recombination event that leads to the μ/α switch (see below). We refer to it as the "switch region." The homology shared between these sequences was confirmed by using *in situ* hybridization with a probe derived from the 5′ portion of the mouse μ clone (the *Xba* fragment). This probe crosshybridizes to the analogous segment of the human gene (Fig. 2, lane A). A probe derived from the mouse coding sequence crosshybridizes to an analogous coding region of the human gene (Fig. 2, lane B).

Comparison of Mouse and Human Germ-Line α Genes. A similar evolutionary comparison can be made between the mouse and human germ-line α genes. A heteroduplex molecule formed between the 13.5-kb mouse segment and the 16-kb human segment is shown in Fig. 1*B*. Its pattern of conserved homology is similar to that between human and mouse μ genes (Fig. 1*A*). The four short segments of homology interrupted by nonhomologous bubbles encompass the coding and intervening sequence segments of the α gene. The fourth homology region is likely to represent a coding segment analogous to the anchor peptide sequence seen in μ chain genes (34). As with the μ genes, approximately 2.0 kb to the 5′ side of the coding region there is an extensive (2.7 kb) region of conserved homology, again encompassing the region of DNA involved in the μ/α switch (see below). Again this conserved switch segment is bordered by regions of nonhomology that evidently escape the selective pressures operating on the coding and switch regions.

Evolutionarily Conserved Switch Regions Share Extensive Homology Between μ and α Genes. Above we demonstrated the evolutionary conservation of an approximately 2-kb segment of DNA that encompasses the recombination site of the μ/α switch. Limited sequence studies of the α/μ recombination site have failed to reveal precise homology or some obvious structural feature [such as that seen in V/J recombination (6, 7)] that suggest a molecular basis for this recombination event. However, heteroduplex comparison of these regions in μ and α genes provides rather dramatic evidence of extensive homology between the switch regions that lie on the 5′ side of the two genes (Fig. 1 *C* and *D*). When the mouse μ and α sequences are compared in this way (Fig. 1*C*), an approximately 2-kb region of homology appears to the 5′ side of each coding segment. Within the rough limits of measurement, this region corresponds to the regions conserved between the human and mouse gene μ and α fragments. Similarly, when human μ and α segments are compared by heteroduplex mapping (Fig. 1*D*) an analogous 2-kb segment of homology is detected. [An additional 0.5-kb segment of homology is also present between the 3′ border of the human μ coding sequence and the 5′ border of the human α coding sequence (see diagram, Fig. 1*D*).]

The extent of homology between μ and α genes is confirmed by *in situ* hybridization with the 5′ *Xba* fragment derived from the mouse μ clone (see diagram, Fig. 2). This probe includes the region of homology identified in the human/mouse heteroduplex studies and readily detects a region of homology on a 4.2-kb *Eco*RI/*Hin*dIII fragment from the mouse α gene (Fig. 2, lane C) (see map in Fig. 3). An analogous probe (an 8-kb *Eco*RI fragment) derived from the human μ clone (see Fig. 2) detects a region of homology in an 11.8-kb *Bam*/*Xho* fragment derived from the human α gene (Fig. 2, lane E). The coding segment for the human α gene which maps to the 3′ side of the switch region is contained within a 4.2-kb *Bam*/*Xho* fragment and hybridizes to a probe derived from the mouse α coding region (Fig. 2, lane F; map, Fig. 3).

Conserved Homology Regions Encompass H Chain Switch Region. In order to establish whether the homology between embryonic μ and α genes might be relevant to the H chain switch, we cloned a rearranged α gene from the S107 cell line that expresses a phosphorylcholine-binding IgA (35). Separate restriction mapping studies (not shown) showed that this clone is a μ/α recombinant, containing α embryonic sequences for approximately 2 kb 3′ to the *Pst* site (diagrammed in Fig. 3) and μ embryonic sequences from the 5′ *Eco*RI site in the μ gene up to and including the second *Hin*dIII site 1.75 kb 3′ to the *Eco*RI site. When probed with a mouse α constant region probe (the *Eco*RI/*Xba* fragment) *Eco*RI/*Hin*dIII digests of a mouse α embryonic clone (Fig. 2, lane G) and the S107 clone (Fig. 2, lane H) demonstrated that a rearrangement occurred. This rearrangement introduces a *Hin*dIII site 3.6 kb to the 5′ side of the *Eco*RI site in the α embryonic gene (see Fig. 3).

We identified this novel *Hin*dIII site in the S107 clone as representing mouse μ embryonic sequences as follows. *Eco*RI/*Hin*dIII digests of the α embryonic clone (Fig. 2, lane C) and the S107 clone (Fig. 2, lane D) were probed with a fragment derived from the 5′ region of the mouse μ embryonic clone (the *Xba* fragment, see Fig. 2). The α embryonic clone crosshybridized to this probe, yielding a 4.2-kb fragment and a second, faint, 1.0-kb fragment, corresponding to the *Eco*RI/*Hin*dIII fragment and the internal *Hin*dIII fragment diagrammed in Fig. 3. S107, however, demonstrates crosshybridization to a 3.6-kb *Eco*RI/*Hin*dIII fragment as well as two additional fragments 0.75 and 0.70 kb long (Fig. 2, lane D; Fig. 3). These two small fragments represent an internal *Hin*dIII fragment (0.70 kb) derived from the μ embryonic gene and an *Eco*RI/*Hin*dIII fragment (0.75 kb) derived from the 5′ end of the μ embryonic clone. We can conclude from this and other similar mapping studies (data not shown) that S107 is a μ/α

Table 1. Summary of clones

Clone	Insert, kb	Source of DNA	Linkage sites	Vector
Mμ	11.2	Mouse embryo	*Eco*RI (GM)	CH4A
Huμ	11	Human fetal liver	*Eco*RI (*Alu*/*Hae* III)	CH4A
Mα	13.5	Mouse embryo	*Eco*RI (*Hae* III)	CH4A
Huα1*	16	Human placenta	*Bam*HI (GM)	CH28
Mγ1	10.5	Mouse embryo	*Eco*RI (*Hae* III)	CH4A
MαS107	5.0	S107 myeloma	*Eco*RI (GM)	CH4A

kb, kilobase; GM, DNA fragment isolated by preparative agarose electrophoresis (30); Hu, human; M, mouse.

* Huα1 identified by nucleotide sequence analysis of the cloned gene.

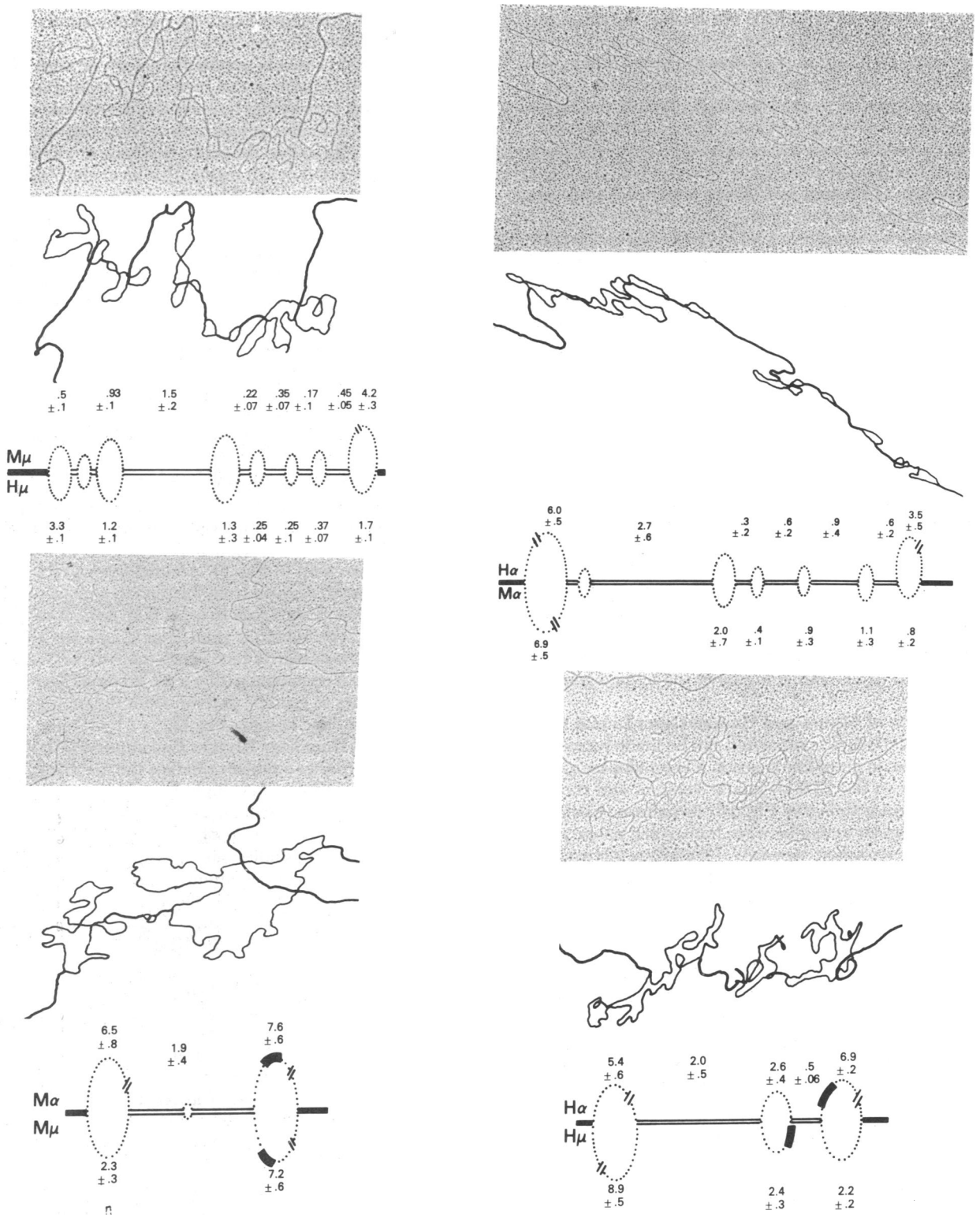

FIG. 1. Heteroduplex analysis of mouse (M) and human (H) H chain genes. Each panel consists of three components: the actual electron micrograph, a line drawing of the electron micrograph, and a diagram identifying various regions of the gene fragments. The numbers above and below the schematic diagram indicate the mean (±SD) of measurements (in kb) of 10 molecules. All molecules are arranged 5′ (left) to 3′ (right). The arms of the phage λ are indicated by dark solid lines at each end of the diagram. (*A*) Human μ/mouse μ. (*B*) Human α/mouse α. (*C*) Mouse μ/mouse α. (*D*) Human μ/human α. Solid blocks indicate the coding regions of the cloned gene fragments.

recombinant, with recombination having occurred between the two *Hin*dIII sites 1.75 and 2.75 kb 3′ to the *Eco*RI site of the embryonic μ sequence. This region is underscored in Fig. 3. The heteroduplex mapping referred to above places this recombination site within the region that shows extensive homology between the μ and α germ-line genes.

Earlier evidence establishing that this region is a general site of H chain switching was provided by Davis *et al.* (10) who showed that the IgA-producing myeloma M603 gene has undergone a recombination event in this same region, between the *Sac* I site (2.25 kb 3′ to the μ *Eco*RI site) and the *Hin*dIII site (2.75 kb 3′ to the μ *Eco*RI site) (Fig. 3). Sakano *et al.* (15)

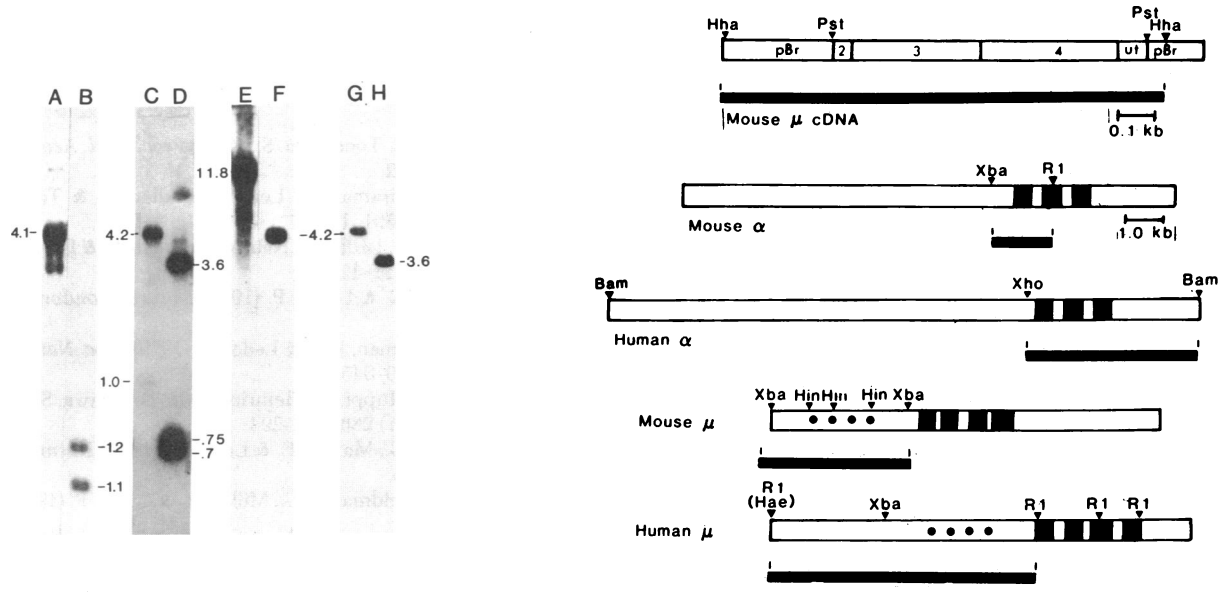

FIG. 2. *In situ* hybridization of human and mouse H chain gene fragments. DNA was prepared from phage λ vectors containing the cloned gene fragments to be analyzed (33). Restriction endonuclease digestion and agarose electrophoresis were followed by transfer to nitrocellulose filters according to the procedure of Southern (27). ^{32}P-Labeled probes were hybridized to the immobilized DNA fragments. Autoradiograms of the blots are shown at *Left;* maps of the probes used are shown at *Right*, with relevant restriction sites. Lanes: A, *Eco*RI/*Xba* digest of the human μ embryonic clone hybridized to the mouse 5′ *Xba* probe (indicated by solid bar under mouse μ map; dotted area identifies the switch region); B, *Eco*RI/*Xba* digest of the human μ embryonic clone hybridized to the mouse μ cDNA probe (indicated by the solid bar under the mouse μ cDNA map); C, *Eco*RI/*Hin*dIII digest of the mouse α embryonic clone hybridized to the mouse 5′ *Xba* probe; D, *Eco*RI/*Hin*dIII digest of the mouse α rearranged clone (MαS107) hybridized to the mouse 5′ *Xba* probe; E, *Bam*/*Xba* digest of the human α embryonic clone hybridized to the human μ 5′ probe (indicated by the solid bar under the human μ map, dotted area signifies the switch region); F, *Bam*/*Xba* digest of the human α embryonic clone hybridized to the mouse α coding region *Eco*RI/*Xba* probe (indicated by the solid bar under the mouse α map); G, *Eco*RI/*Hin*dIII digest of the mouse α embryonic clone hybridized to the mouse α coding region *Eco*RI/*Xba* probe; H, *Eco*RI/*Hin*dIII digest of the mouse α rearranged clone (MαS107) hybridized to the mouse α coding region *Eco*RI/*Xba* probe.

and Takahashi *et al.* (14) also showed that γ2B DNA rearrangements occur within this 2-kb region of the mouse μ embryonic gene. Kataoka *et al.* (11) showed that a rearrangement generating a γ1 gene has occurred within this same 2-kb embryonic μ region. It appears, then, that the region involved in heavy chain switching from μ to α and from μ to γ is included in the homology region we find shared by the μ and α germ-line genes and conserved in mouse and man.

DISCUSSION

Evolutionary Conservation of H Chain Switch Region: Possible Role for Sequence Homology in μ/α Switch. Evolution has conserved a ≈2-kb segment of DNA that represents a major region of homology between the μ and α gene sequences (summarized in Fig. 4). Moreover, this segment covers the region involved in all the μ/α and μ/γ (see below) switches thus far analyzed (refs. 10, 11, 13–15; M. Davis, S. Kim, and L. Hood, personal communication). The extensive homology observed between these segments in μ and α genes provides a possible basis for the recombination event that mediates the μ/α switch. The nucleotide sequence of the mouse μ switch region reveals a striking series of internally reiterated sequences (14, 15). Specifically, the pentanucleotide T-G-A-G-C is repeated 40 times, both in tandem arrays and as isolated units. Permutations of this pentanucleotide are also highly repeated. In addition, a higher-order repeat, $(T\text{-}T\text{-}G\text{-}A\text{-}G\text{-}C)_3G\text{-}G\text{-}G$, is repeated four times. These repeated elements are clustered in the 1.0-kb region between the *Hin*dIII sites 1.75 and 2.75 kb 3′ from the mouse μ *Eco*RI site (Figs. 2 and 3). Our sequence data from

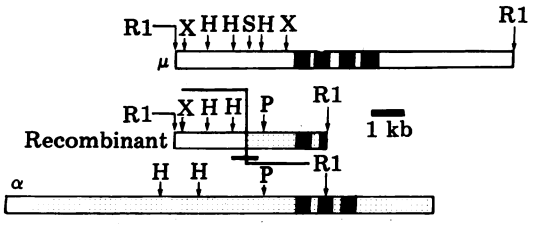

FIG. 3. Diagram of germ-line mouse μ and α genes and active α gene formed by recombination. The recombined gene is that derived from the IgA myeloma S107 (35). Relevant restriction sites for each gene fragment are shown (R1, *Eco*RI; X, *Xba;* H, *Hin*dIII; S, *Sac* I; P, *Pst* I). Coding regions are indicated by solid blocks. The mouse μ gene fragment is indicated by an open box and the mouse α gene fragment is indicated by a stippled box. A solid line under the recombinant gene fragment indicates the location of the recombination event.

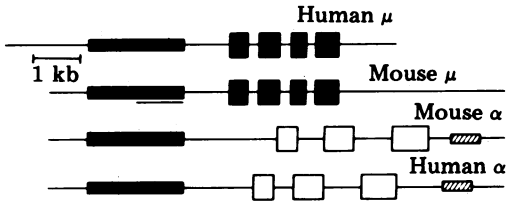

FIG. 4. Homologies conserved among human and mouse H chain genes. The long thick bar indicates the ≈2 kb of conserved homology among the various genes studied, which spans the H chain switch region. Four solid squares and rectangles indicate the homology conserved in the coding regions of the human and mouse μ genes; the open blocks indicate the conserved homology which includes the human and mouse α gene coding regions. The smaller, hatched blocks indicate a region of homology between the human and mouse α genes which lies 3′ to the third domain. (This segment might encode a membrane anchor peptide.) The thin line indicates the site of recombination for the mouse rearranged α gene derived from the S107 myeloma.

a cross-hybridizing region of the mouse α gene (unpublished data) reveal the same pentanucleotide element multiply repeated, as well as the higher order repeat $(T\text{-}G\text{-}A\text{-}G\text{-}C)_3G\text{-}G\text{-}G$.

Such data suggest that these repeating elements can maximize imperfect homology between these two genes and might provide a large, although imperfect, target for homologous recombination. Nevertheless, this μ/α recombination may not involve homologous recombination as it is rigorously defined. The single limited example of a μ/α recombination site of known sequence does not reveal homology between the recombined DNA segments (M. Davis, S. King, and L. Hood, personal communication). Therefore, we must consider the possibility that the extensive homology we observe provides a rough guide (for example, a recombinant heteroduplex structure) for a specialized recombination system that cleaves and joins at a nearby bubble of nonhomology. In any case, it is still possible that the switch reaction relies on specialized, rather than general, recombination mechanisms.

μ/γ Switch. More recent studies (11, 13–15) in the mouse have shown that the μ/γ switch analogous to that described for the α switch (10). In the published examples (11, 13–15), both $\gamma 1$ and $\gamma 2b$ rearrangements involve sites within the same 2-kb switch sequence that we find conserved between mouse and man. One can logically ask whether similar homology regions flank the germ-line γ genes. Utilizing the switch region fragment from the human μ clone as a probe, we have been able to select multiple human clones from a placental library that crosshybridize to mouse α constant region probes. To date, we have not been able to select any γ crosshybridizing clones by this approach. In preliminary experiments, we have tested many mouse and human γ clones for their ability to crosshybridize to probes containing the μ switch region. We have not detected any homology. Although it is possible that some of our clones do not extend far enough 5′ to the coding region to include the γ switch site, this is very unlikely in at least one mouse $\gamma 1$ gene we have analyzed because it retains 3–4 kb of sequence to the 5′ side of the coding region including the μ/γ switch site as mapped by Kataoka *et al.* (11). Alternatively, as has been noted (10–15, 31, 32), the μ gene undergoes deletion upon cloning. Indeed, we have isolated an 11.2-kb mouse μ *Eco*RI fragment that is 1 kb shorter than the 12.2-kb genomic *Eco*RI fragment from mouse embryonic DNA. We cannot rule out the possibility, therefore, that this deleted 1-kb region includes a region of μ/γ homology.

We must also consider the possibility that different classes of H chains use different structural signals and enzyme systems to catalyze their respective switches. One attractive notion would be to imagine that the γ switch signal does not rely upon homology but uses sites within the μ/α homology region that eliminate a considerable segment of this region during the μ/γ switch. The γ recombinant would now lack a major segment of the μ/α switch region and would be unlikely to undergo further recombination. Of course, it is equally possible to imagine that μ/γ switch involves homology recognition at some alternative site (not included in our clones) or much smaller homologous recognition sequences such as the small palindromic sequences thought to be involved in V/J recombination (6).

We are grateful to Alfred Bothwell, David Baltimore, and Tasku Honjo for their generous gifts of mouse α and $\gamma 1$ cDNA clones, to J. G. Seidman for providing the mouse embryo library and to Tom Maniatis for the human fetal liver library used in these studies, to Jake Maizel for his computer analysis of these data, to Barbara Norman and Marion Nau for their invaluable help in the creation of the mouse and human clone libraries, and to Terri Broderick for her skilled assistance in the preparation of the manuscript.

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