Proc. Natl. Acad. Sci. USA
Vol. 77, No. 2, pp. 1106–1110, February 1980
Immunology

Organization of κ light chain genes in germ-line and somatic tissue

(antibody diversity/germ-line genes/restriction map)

ROLF JOHO*, IRVING L. WEISSMAN*, PHILIP EARLY†, JIM COLE†, AND LEROY HOOD†

*Laboratory of Experimental Oncology, Department of Pathology, Stanford University, Stanford, California 94305; and †Division of Biology, California Institute of Technology, Pasadena, California 91125

Communicated by H. N. Eisen, October 5, 1979

ABSTRACT **We studied the organization of the κ light chain genes in germ-line (sperm) and somatic (embryo) tissues. We constructed a plasmid containing a DNA insert coding for the κ chain MOPC 167 and used the Southern blotting technique to determine the organization of κ variable and constant region genes. In the haploid genome of the mouse there is only one constant region gene detectable and it has the same organization in sperm and embryo DNAs. There are several variable region genes in sperm and embryo that are related to the V_{k167} gene. The organization of the V genes in sperm and embryo DNAs is identical. These results show that there is no rearrangement of variable region genes (or "minigenes") during early embryogenesis.**

The polypeptides that form antibody molecules can be divided into two distinct parts, the variable (V) and the constant (C) region. From RNA·DNA hybridization studies, only a few copies of a particular C region seem to be present in the genome (1–11). The number of V region genes present in the germ line is still not resolved. Because the V regions determine the antibody specificity, the question of the origin of antibody diversity is an integral part of the number and organization of the V region genes. Several hypotheses have been proposed to explain the origin of antibody diversity. The strict germ-line hypothesis proposes that there are multiple V region genes and each antibody-producing cell chooses to express only one of these genes from the inherited V region repertoire (12). The various somatic diversification hypotheses explain the origin of antibody diversity quite differently. It is assumed that the germ line contains only a few V region genes and these are somatically diversified during ontogeny in order to yield many different V regions. A strict mutation hypothesis proposes that mutations (simple base changes) occur during differentiation of precursor cells into antibody-producing cells (13, 14). These mutations may occur in the hypervariable part of the V region or they may occur randomly throughout the V region gene. The somatic recombination hypothesis postulates a limited number of V genes in the germ line which undergo unequal crossing-over during ontogeny to form recombined V genes, thereby expanding the V region information that is expressed (15, 16).

Wu and Kabat (17) pointed out that amino acid sequence analysis of several hundred light chain V regions suggested a division of V regions into four "framework" regions with very little variability (FR1, FR2, FR3, and FR4) and three hypervariable regions (HV1, HV2, and HV3) that exhibit extensive sequence diversity. Kabat and others later proposed that each of these V region segments was a germ-line minigene that was inherited independently (18–20). By these hypotheses, subgenic elements representing all possible V region framework and hypervariable sequences would be inherited in the germ line and would be rearranged during ontogeny to create diverse V region coding sequences. One version of this model postulates that the DNAs coding for the hypervariable segments are inserted into a preexisting framework region of the V gene (17). Other somatic rearrangements of inherited "minigene" V region fragments have been proposed (18–20), and it is possible to develop several models that would predict expansion, contraction, or insertion of hypervariable regions.

However, two groups recently cloned and characterized mouse immunoglobulin light chain V region genes, and their results place serious constraints on the minigene hypothesis. Seidman *et al.* (21, 22) cloned and determined the sequence of two κ light chain V region genes from neoplastic antibody-producing cells (myelomas). Tonegawa *et al.* (23, 24) cloned and determined the sequence of a λ V region gene from embryonic DNA, assuming this to be representative of germ-line DNA. In contrast to the minigene hypothesis, they found an intact V region containing, in order, FR1-HV1-FR2-HV2-FR3-HV3. Although FR4 was present in 12-day embryo DNA as a noncontiguous genetic element, consistent with part of the minigene hypothesis, the fact that the HV regions were in place ruled out HV insertions at a later stage in development. However, it was still possible that gene insertion or gene reassortment events could occur prior to day 12 of embryogenesis and that some other organization of V region minigenes exists and is inherited via germ-line DNA.

We think that, in order to make any conclusions as to the origin of antibody diversity, it is necessary to study the gene organization in the germ line. For this reason we have characterized the organization of mouse κ light chain genes in sperm and embryo DNA. To do this we constructed a plasmid containing a DNA insert coding for the κ light chain MOPC 167 and used the Southern blotting technique to determine the organization of κ V and C region genes. This analysis demonstrates unequivocally that the V_{k167} gene family is not altered in size from germ line to 15-day embryos and, therefore, places serious constraints on minigene hypotheses that would require insertions, expansions, or contractions of V_k genes during embryogenesis.

MATERIALS AND METHODS

Preparation of Light Chain mRNA. Membrane-bound polysomal poly(A)$^+$ RNA was prepared from MOPC 167 tumors (25) and fractionated on an isokinetic 10–40% sucrose gradient. The peak of light chain mRNA was identified by *in vitro* translation in rabbit reticulocyte lysate (26).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: V, variable; C, constant; bp, base pair(s); kb, kilobase(s).

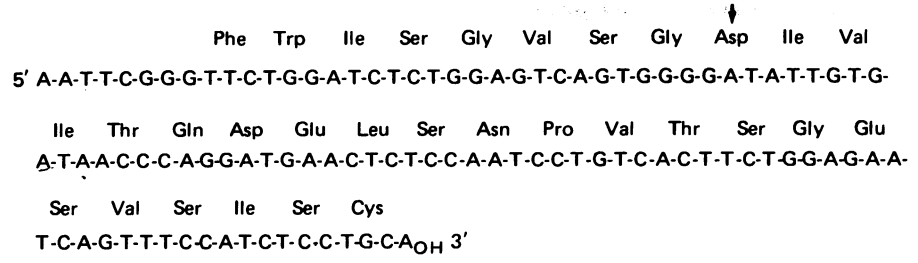

FIG. 1. DNA sequence of the 100-bp *Eco*RI/*Pst* I fragment with the corresponding amino acid sequence of MOPC 167 κ chain (38). The arrow indicates the amino terminus of the mature light chain.

cDNA Cloning. Double-stranded cDNA was synthesized from MOPC 167 light chain mRNA (27). The cDNA (0.5–1 μg) was incubated in 25 μl of 60 mM Tris·HCl, pH 7.5/8mM $MgCl_2$/4mM dithiothreitol/0.2 mM deoxynucleotide triphosphates with 5 units of *Escherichia coli* polymerase I (Boehringer Mannheim) at 12°C for 10 min to produce flush ends (28). Synthetic *Eco*RI dodecamer linkers (gift of R. Scheller) and the cDNA were phosphorylated as described (29) and ligated to 0.5 μg of *Eco*RI-digested pMB9. The mixture was used directly to transform *E. coli* 1776 as described (30). Transformants containing light chain sequences were identified by using the Grunstein–Hogness procedure (31) with kinase ^{32}P-labeled MOPC 167 light chain mRNA for hybridization (32).

Purification of Mouse Sperm. BALB/c mouse sperm was purified from vas deferens and epididymis. The tissue was minced with a razor blade and suspended in medium 199 containing 5% fetal calf serum for 15–30 min at room temperature. The supernatant was removed and the pelleted tissue fragments were resuspended in the same medium. After centrifugation (1 min at 1000 rpm), both supernatants were combined and freed from contaminating tissue by filtration through a nylon screen. The spermatocytes were pelleted by centrifugation for 10 min at 2500 rpm and contaminating somatic cells were removed by lysis in 1.5% sarcosinate according to Witkin *et al.* (33). Microscopic inspection of the sperm suspension revealed no detectable contamination with somatic cells (less than 1 nucleated cell per 200 spermatocytes).

Preparation of Genomic DNA. Mouse sperm cells were lysed in the presence of 2% $NaDodSO_4$ and 0.14 M 2-mercaptoethanol and digested with proteinase K (0.5 mg/ml) at 50°C for 3 hr. Sperm DNA and BALB/c embryo DNA (15 days' gestation) were prepared according to Blin and Stafford (34).

Agarose Gel Electrophoresis and Transfer to Nitrocellulose Filter Paper. Mouse DNA (10 μg) digested with restriction endonucleases (enzymes were obtained from Bethesda Research Laboratories) was electrophoresed through horizontal agarose gels in 70 mM Tris·HCl, pH 7.8/60 mM Na phosphate/2 mM EDTA at room temperature. The DNA was transferred to nitrocellulose filters as described by Southern (35). The filters were hybridized to nick-translated ^{32}P-labeled DNA (36). If not otherwise stated, the filters were given a final wash in 45 mM NaCl/4.5 mM Na citrate/0.1% Na pyrophosphate/0.1% $NaDodSO_4$ and exposed for 4–14 days on Kodak X-Omat XR5 film with a Dupont intensifying screen at −70°C.

Preparation of V_k and C_k Probes. *Hin*cII-digested plasmid p167kRI was electrophoresed through 1.2% agarose in 40 mM Tris acetate, pH 7.7/1 mM EDTA/ethidium bromide (0.5 μg/ml). The DNA fragments containing V_k or C_k sequences were electro-eluted according to Tabak and Flavell (37) except that hydroxyapatite in the trough was replaced by DEAE-cellulose (Whatman DE-52) suspended in electrophoresis buffer. The eluted DNA was loaded onto a 0.2-ml DEAE-cellulose column and the DNA fragment was eluted with 0.6 M NaCl/50 mM Tris·HCl, pH 7.5/1 mM EDTA, precipitated with ethanol, and used for nick translation.

RESULTS

The Plasmid p167kRI Contains a Complete κ Light Chain Insert. The plasmid p167kRI was found to contain an *Eco*RI-excisable insert of approximately 900 base pairs (bp). To determine what portion of the mRNA sequence was contained in this plasmid, it was cut with *Eco*RI, and the 900-base-pair insert was purified and end-labeled with [^{32}P]phosphate by use of polynucleotide kinase. The labeled insert was digested with either *Pst* I or *Hin*cII to produce terminally labeled fragments. The DNA sequence of the 100-base-pair *Eco*RI/*Pst* I fragment was determined according to Maxam and Gilbert (32) (Fig. 1). This sequence contains nucleotides corresponding to the amino terminus of the κ light chain sequence of MOPC 167 (38). Adjacent to the terminal linker oligonucleotide there are an additional 25 nucleotides preceding the sequence coding for the amino terminus of the κ light chain. These nucleotides presumably code for part of the "signal peptide" removed *in vivo* from the primary translation product (39).

Digestion of the end-labeled κ chain insert with *Hin*cII yielded two terminally labeled fragments of approximately 310 and 470 bp, respectively. A partial sequence of 80 nucleotides of the 470-bp *Hin*cII fragment corresponded to the 3′ untranslated region sequence of mouse mRNA determined by Hamlyn *et al.* (40), beginning five nucleotides from the 3′ end [excluding the poly (A)] (results not shown).

In order to orient the κ chain insert in pMB9 we digested the recombinant plasmid with *Hin*cII. This generated three fragments of 2800, 2200, and 1300 bp, respectively. In pMB9, the two *Hin*cII sites are 910 and 2350 bp from the single *Eco*RI site into which the 900 bp κ light chain sequence was inserted (41). From these data we can conclude that the 5′ terminal part of the insert is oriented toward the *Hin*cII site closest to the *Eco*RI site, whereas the 3′ untranslated part of the κ gene insert is oriented toward the *Hin*cII site farther away from the *Eco*RI site.

From the DNA sequence data of Hamlyn *et al.* (40) it is evident that the *Hin*cII site at amino acid position 125 coincides with an *Hpa* I site and that there is a *Hae* III site at amino acid position 196. The purified κ chain insert was found to contain one *Hpa* I and one *Hae* III site at the corresponding amino acid positions 125 and 196, respectively. Fig. 2 shows a restriction map of p167kRI.

Organization of κ Light Chain Genes in Germ Line and Embryo. In order to compare the organization of the κ light chain genes in germ-line and somatic tissue, BALB/c sperm and embryo DNA were digested with *Bam*HI, *Bgl* II, *Eco*RI, and *Hin*dIII. These enzymes do not cleave the κ chain insert in p167kRI. The DNA fragments were separated by electrophoresis, transferred to nitrocellulose filter paper, and hybridized to ^{32}P-labeled plasmid p167kRI containing V_{k167} and C_k sequences. The same band pattern was obtained when sperm or embryo DNA was digested with a particular restriction enzyme (Fig. 3*A*). With each type of restriction digest, a very strong band was visible in addition to a few weak ones. In order to clarify the origin of strong and weak bands, separate ^{32}P-labeled probes specific for V_{k167} and C_k sequences were prepared and used in all subsequent experiments.

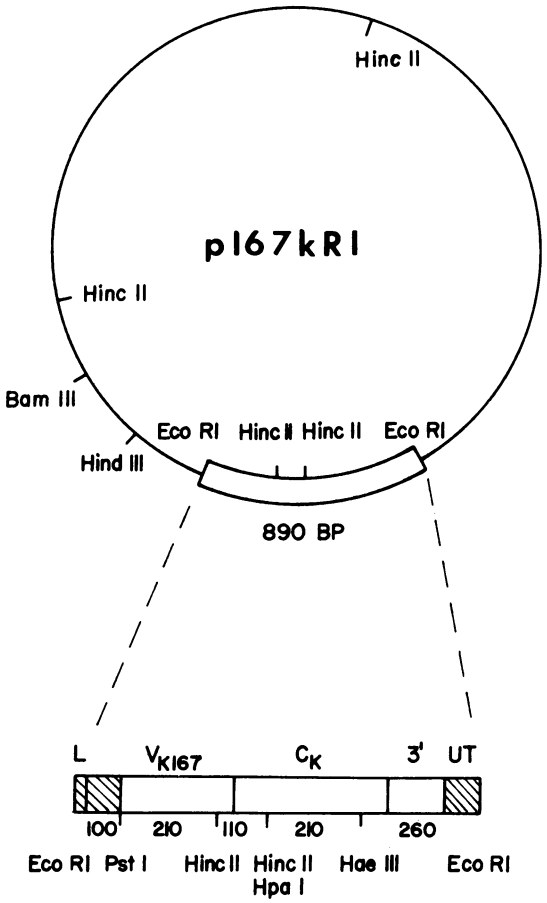

FIG. 2. Restriction map of p167kRI. The hatched area of the insert represents DNA for which the sequence has been determined. There is one additional *Pst* site at amino acid 43 (not shown on map).

Organization of Region Surrounding C_k Gene. The experiment described above was repeated but instead of plasmid p167kRI, a ^{32}P-labeled DNA fragment containing C_k sequences corresponding to the sequence from amino acid 125 to the carboxy terminus (including the 3′ untranslated region) was used as a probe for hybridization (Fig. 3*B*). The faint bands visible in the first experiment were no longer detected, and only one intense band corresponding in position to the intense one in the above experiment could be seen. Sperm and embryo DNAs yielded the same size fragment when digested with a particular restriction enzyme. The lengths of the fragments were 13, 2.7, 17, and 4.3 kilobases (kb) when digested with *Bam*HI, *Bgl* II, *Eco*RI, and *Hin*dIII, respectively.

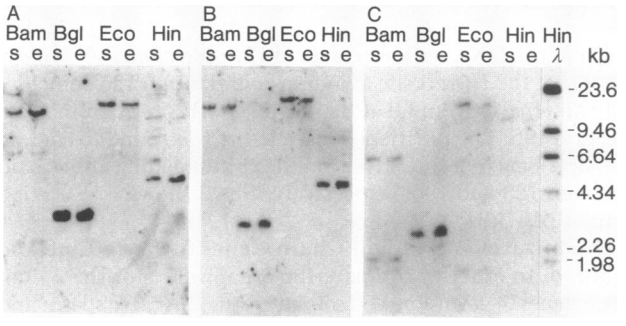

FIG. 3. Sequences in restriction endonuclease-digested BALB/c DNA. BALB/c sperm (lanes s) and embryo (lanes e) DNA were digested with *Bam*HI, *Bgl* II, *Eco*RI, and *Hin*dIII. The resulting fragments were electrophoresed through 0.7% agarose, transferred to nitrocellulose filter paper, and hybridized to the nick-translated plasmid p167kRI. *Hin*dIII-digested bacteriophage λ DNA was used as a size marker and nick-translated λ DNA was included during the hybridization. The *Hin*dIII-digested sperm DNA shows some partial digestion products. (*A*) V_k and C_k sequences. (*B*) C_k sequences; the plasmid p167kRI was replaced with a C_k specific probe. (*C*) V_{k167} related sequences; a V_{k167} specific probe was used during hybridization.

In order to locate the positions of the various restriction sites near the C_k sequences in the mouse genome, we performed double digestions on BALB/c sperm and embryo DNAs and analyzed the DNA digests with a C_k specific probe (Fig. 4*A*). We conclude that the organization of the C_k region is the same in the germ line as in the embryo (as far as one can tell by mapping several restriction sites). These results also permit us to construct a map of the restriction sites flanking the C_k sequence (Fig. 5). The C_k sequences are located on a 1.8-kb fragment terminated by *Hin*dIII and a *Bgl* II site. If we calculate 321 bp for the coding part of the C_k gene and 211 bp for the 3′ untranslated region (40), we can expect 532 bp for the entire C_k region gene (provided that there are no intervening sequences present). Thus, the 1.8-kb *Hin*dIII/*Bgl* II fragment could contain as many as three C_k genes, which is compatible with DNA·RNA hybridization data (1–10).

Only One C_k Gene Is Present in the Mapped Region of the Genome. In order to determine how many C_k region genes are present in the germ line, we took advantage of the fact that there is an *Hpa* I site at the codon for amino acid 125. This *Hpa* I site is therefore close to the beginning of the C_k gene (close to the V–C junction). By mapping this *Hpa* I site it should be possible to locate any other C_k genes within the 1.8-kb *Hin*dIII/*Bgl* II restriction fragment.

Hpa I-digested sperm and embryo DNA were further digested with *Bam*HI, *Bgl* II, or *Hin*dIII and analyzed with the C_k specific probe (Fig. 4*B*). The DNA sequence coding for the constant part of the κ chain was in a 0.5-kb segment flanked on one side by the *Hpa* I site and on the other side by the *Bgl* II site (Fig. 5). The orientation of transcription was determined by the known position of the *Hpa* I site in the amino acid coding sequence. These experiments show that there is only one C_k gene in the region mapped in germ line and embryo DNAs. The possibility that the entire region shown in the restriction map may be repeated in the genome cannot be eliminated.

Organization of V_k Genes. Because the intense bands in Fig. 3*A* corresponded to the fragment carrying the C_k region, it was reasonable to assume that all the faint bands visible contained

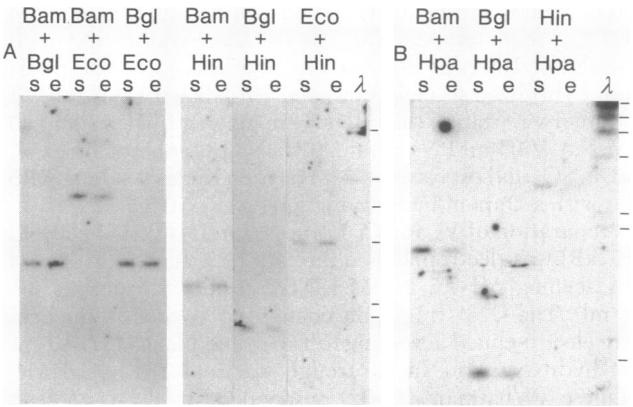

FIG. 4. Sperm (lanes s) and embryo (lanes e) DNAs were subjected to two consecutive digestions as indicated. Analysis was as described in Fig. 3*B*.

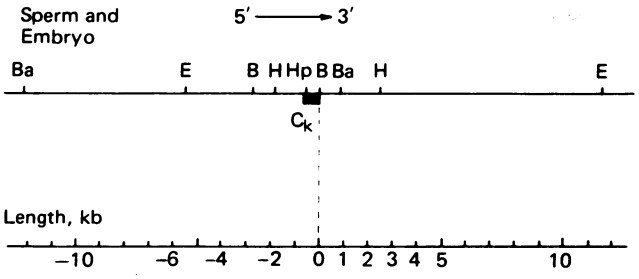

FIG. 5. Map of several restriction sites flanking the germ-line C_k gene. The C_k gene including the 3′ untranslated region is indicated by black box. The location of several restriction sites around the C_k gene is shown and drawn to scale. On either side of the C_k gene, only the nearest restriction site of a particular enzyme is shown. The arrow indicates the orientation of the C_k gene (direction of transcription). Ba, *Bam*HI; B, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa* I.

V_k sequences related to or identical with the V region sequence expressed in MOPC 167. In order to test this assumption, the same experiment as described in Fig. 3*B* was done except that the C_k probe was replaced by a V_{k167} probe (including the V region sequence from the amino terminus to amino acid 93) (Fig. 3*C*). The same faint bands were visible, and sperm and embryo DNAs revealed the same band patterns when analyzed with any particular restriction enzyme. Although the strong band containing the C_k gene had disappeared for the most part, it was still visible to a varying degree. The V_{k167} probe used in these experiments was slightly contaminated by C_k probe; even a small contamination of the V_{k167} probe with C_k probe shows in this type of analysis. We therefore think that the bands visible at the C_k position when a V_{k167} probe was used were due to contaminating C_k sequences in the probe. However, the possibility that a V_k gene is close to the C_k gene or present on a restriction fragment of similar size cannot be ruled out.

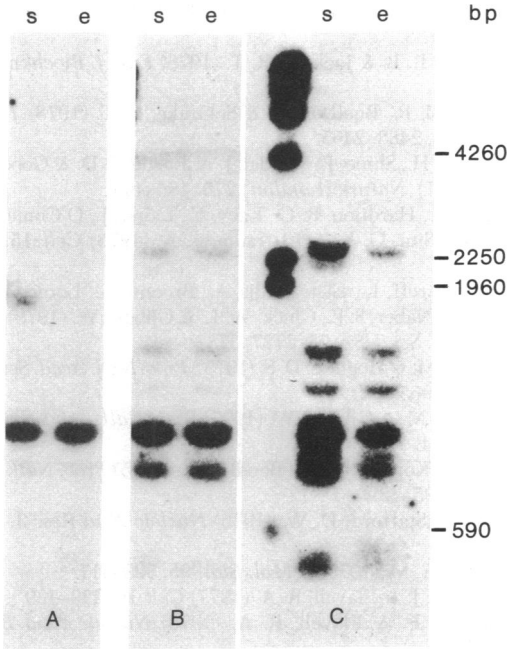

FIG. 6. V_{k167} related sequences in *Hae* III-digested BALB/c DNA. Sperm (lanes s) and embryo (lanes e) DNAs were digested with *Hae* III and the resulting fragments were electrophoresed through 2% agarose. The V_{k167} related sequences were analyzed as described in Fig. 3, except that the filters were washed under various salt conditions: *A*, 15 mM NaCl/1.5 mM Na citrate; *B*, 45 mM NaCl/4.5 mM/Na citrate; *C*, 0.3 M NaCl/0.03 M Na citrate. The right two lanes in *C* show some differences in band intensity but not in band pattern.

The band patterns of sperm and embryo DNA restriction digests appeared to be identical. Because the various restriction fragments carrying V_{k167} related sequences were large (up to several kilobases), it would have been difficult to detect small size differences (which could be involved in rearrangements or insertion of hypervariable subgenic elements). In order to solve this problem we used a restriction enzyme that produced smaller fragments but did not cut the V region V_{k167} of the plasmid (Fig. 2). Surprisingly, a simple picture emerged when *Hae* III digests of sperm and embryo DNAs were analyzed with the V_{k167} probe (Fig. 6). Instead of finding several smaller bands corresponding to as many larger bands that could be seen before, only two bands were found when the filters were washed under the same salt conditions (45 mM NaCl/4.5 mM Na citrate). Under more stringent washing conditions, only one band of 950-bp was seen, whereas under less stringent conditions (0.3 M NaCl/0.03 M Na citrate) as many as seven bands could be detected. None of these bands was due to C_k sequences because a similar analysis with the C_k probe yielded two bands of different length (results not shown). All the bands had the same position in sperm and embryo DNAs. This experiment therefore shows clearly that there is no detectable difference in the gene organization for V_{k167} related V region genes between germ-line and embryo tissues. In this experiment, size differences as small as 20 bp would easily have been detected, ruling out major size rearrangements of the V_{k167} related V region genes during embryogenesis. Whether the germ-line and embryo genes are different or not in their DNA sequences cannot be decided from the data presented here.

DISCUSSION

Because immunoglobulin genes from embryo and myeloma DNAs have been cloned and analyzed, we thought that it was important to compare the gene organization in the germ line to that in these somatic tissues. We used the Southern blotting technique to analyze V_{k167} and C_k sequences. A map of several restriction sites flanking the C_k region gene was established and shown to be the same for sperm and embryo. Therefore, one can conclude that during embryogenesis the C_k gene retains its germ-line organization as far as the flanking restriction sites are concerned. From RNA·DNA hybridization studies it was clear that there were only a few (one to four) C_k genes present in the genome, although the exact number was not known (1–11). The results presented here imply that the C_k gene including the 3′ untranslated region is present only once in the haploid mouse genome, unless the entire 23-kb unit shown in Fig. 5 is repeated a few times. Because the C_k gene does not comprise more than about 500 bp, it is clear that large intervening sequences cannot be present.

Analysis of the V region gene(s) was less straightforward. Multiple restriction fragments that hybridized to the V_{k167} probe were detected. These results can be interpreted that the mouse haploid genome contains several κ V region genes that are related to the V_{k167} gene which is expressed in MOPC 167. A similar finding has been reported for V region genes related to the κ light chain gene expressed in MOPC 149 and MOPC 41 (21, 22). Yet there is a remarkable feature about the V_{k167} related V region genes. A V_{k167} probe yielded only very faint bands on a Southern blot compared to a strong signal obtained with a C_k probe. Although it is somewhat difficult to interpret band intensities on a Southern blot (it depends on transfer efficiency of the DNA, specific activity of the probe, length of the probe available for hybridization, etc.) it is a reasonable assumption to expect a restriction fragment carrying a V_{k167}

gene to yield a signal of similar intensity as a restriction fragment bearing the C_k gene. Therefore, the results found (Fig. 3A) when a ^{32}P-labeled probe of the same specific activity for V_{k167} and C_k was used may indicate the absence of a V region gene identical to the one expressed in MOPC 167.

When *Hae* III digests of sperm and embryo DNAs were analyzed for the V region genes only two major bands could be detected with a length of 780 and 950 bp, respectively, Recently, Seidman *et al* (21, 22) reported that the flanking sequences of two cloned related κ V region genes are similar. If related V region genes have similar or identical flanking sequences, then one would expect restriction sites to be conserved within these sequences. Therefore, cleavage with *Hae* III may yield multiple DNA fragments of the same length carrying related V_k genes.

Over the years, several types of hypotheses have been put forward to explain antibody diversity by somatic recombination. Wu and Kabat (17) proposed that DNA segments coding for the hypervariable regions are joined to framework segments in assembling an intact V region. From the work of Tonegawa *et al.* (24) and Seidman *et al.* (21, 22) we know that the V region genes in embryo DNA contain sequences already coding for the hypervariable regions but lack sequences coding for the fourth framework. Therefore, an episomal insertion mechanism as suggested by Wu and Kabat (17) or a gene interaction hypothesis as proposed by Kindt and Capra (19) would have to occur during early embryogenesis. The results shown in Fig. 6 indicate that this does not happen because sperm and embryo DNAs yield the same V_k carrying fragments after *Hae* III restriction. It is still possible that hypervariable insertions could replace germ-line fragments of the same size. We consider these possibilities to be unlikely; the cloning and sequencing of these germ-line genes will resolve this question.

Edelman and Gally (16) suggested that the diversity of V region genes is created by somatic recombination of a few germ-line genes. Recently, Kabat *et al.* (18, 20) presented evidence that this might be the case. Amino acid sequence comparison of more than 500 light chains indicated that the different framework parts of the V region genes might be inherited independently and be put together as a functional V region gene during cell differentiation (early embryogenesis or B-cell differentiation). The fact that V genes in embryo DNA consist only of the V region through HV3 is in accord with this hypothesis. If assembly of V genes happened during embryogenesis, one would expect different restriction patterns of sperm and embryo DNAs. Because we do not find any differences between sperm and embryo DNAs after restriction and Southern blotting, we consider somatic recombination during embryogenesis to be a very unlikely event. Whether somatic recombination happens during B-cell differentiation remains unclear.

This work was supported by American Cancer Society Grant IM56 (to I.L.W.) and National Science Foundation Grant PCM 76-81542 (to L.H.). I.L.W. is a Faculty Research Awardee of the American Cancer Society. R.J. is Postdoctoral Fellow of the Swiss National Foundation. P.E. is supported by National Institutes of Health Training Grant GM 07616.

1. Faust, C. H. Diggelmann, H. & Mach, B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2491–2495.
2. Honjo, T., Packman, S., Swan, D., Nau, M. & Leder, P. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3659–3663.
3. Leder, P., Honjo, T., Packman, S., Swan D., Nau, M. & Norman, B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 5109–5114.
4. Stavnezer, R., Huang, R. C. C., Stavnezer, E. & Bishop, J. M. (1974) *J. Mol. Biol.* **88**, 43–63.
5. Tonegawa, S., Steinberg, C., Dube, S. & Bernardini, A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4027–4031.
6. Rabbitts, T. H. & Milstein, C. (1975) *Eur. J. Biochem.* **52**, 125–133.
7. Rabbitts, T. H., Jarvis, J. M. & Milstein, C. (1975) *Cell* **6**, 5–12.
8. Farace, M. G., Aellen, M. F., Briand, P. A., Faust, C. H., Vasalli, P. & Mach, B. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 727–731.
9. Honjo, T., Packman, S., Swan, D. & Leder, P. (1976) *Biochemistry* **15**, 2780–2785.
10. Tonegawa, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 203–207.
11. Valbuena, O., Marcu, K. B., Weigert, M. & Perry, R. P. (1978) *Nature (London)* **276**, 780–784.
12. Hood, L., Campbell, J. H. & Elgin, S. C. R. (1975) *Annu. Rev. Genet.* **9**, 305–353.
13. Brenner, S. & Milstein, C. (1966) *Nature (London)* **211**, 242–243.
14. Weigert, M., Cesari, I. M., Yonkovich, S. J. Cohn, M. (1970) *Nature (London)* **228**, 1045–1047.
15. Smithies, O. (1963) *Nature (London)* **199**, 1231–1236.
16. Edelman, G. M. & Gally, J. A. (1967) *Proc. Natl. Acad. Sci. USA* **57**, 353–357.
17. Wu, T. T. & Kabat, E. A. (1970) *J. Exp. Med.* **132**, 211–250.
18. Kabat, E. A., Wu, T. T. & Bilofsky, H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2429–2433.
19. Kindt, T. J. & Capra, T. D. (1978) *Immunogenetics* **6**, 309–321.
20. Kabat, E. A., Wu, T. T. & Bilofsky, H. (1979) *J. Exp. Med.* **149**, 1299–1313.
21. Seidman, J. G., Leder, A., Marshall, H. E., Polsky, F., Tilghman, S., Tiemeier, D. & Leder, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3881–3885.
22. Seidman, J. G., Leder, A., Nau, M., Norman, B. & Leder, P. (1978) *Science* **202**, 11–17.
23. Tonegawa, S., Brack, C., Hozumi, N. & Schuller, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3518–3522.
24. Tonegawa, W., Maxam, A. M., Tizard, R., Bernard, O. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1485–1489.
25. Marcu, K. B., Valbuena, O. & Perry, R. P. (1978) *Biochemistry* **17**, 1723–1733.
26. Pelham, H. R. B. & Jackson, R. T. (1976) *Eur. J. Biochem.*, **67**, 247–256.
27. Wickens, M. P., Buell, E. N. & Schimke, R. T. (1978) *J. Biol. Chem.* **253**, 2483–2495.
28. Seeburg, P. H., Shine, J., Martial, J. A. Baxter, J. D. & Goodman, H. M. (1977). *Nature (London)* **270**, 486–494.
29. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstradiadis, A. (1978) *Cell* **15**, 687–701.
30. Villa-Komaroff, L., Efstratiadis, A., Broome, S., Lomedico, P., Tizard, R., Naber, S. P., Chick, W. L. & Gilbert, W. (1978). *Proc. Natl. Acad. Sci. USA* **75**, 3727–3731.
31. Grunstein, M. & Hogness, D. S. (1975). *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
32. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
33. Witkin, S., Korngold, G. & Bendich, A. (1975) *Proc. Natl. Acad. Sci.* **72**, 3295–3299.
34. Blin, N. & Stafford, D. W. (1976) *Nucleic Acid Res.* **3**, 2303–2308.
35. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
36. Jeffreys, A. J. & Flavell, R. A. (1977) *Cell* **12**, 429–439.
37. Tabak, H. F. & Flavell, R. A. (1978) *Nucleic Acid Res.* **3**, 2321–2332.
38. Rudikoff, S. & Potter, M. (1978) *Biochemistry* **17**, 2703–2707.
39. Burstein, Y. & Schechter, I. (1978) *Biochemistry* **17**, 2392–2400.
40. Hamlyn, P., Brownlee, G., Cheng, C., Gait, M. & Milstein, C. (1978) *Cell* **15**, 1067–1075.
41. Tait, R. C. & Boyer, H. W. (1978) *Mol. Gen. Genet.* **164**, 285–288.