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Cloning of immunoglobulin kappa light chain genes from mouse liver and myeloma MOPC 173

Michael Steinmetz*, Hans G.Zachau* and Bernard Mach+

*Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, GFR, and +Department of Microbiology, University of Geneva, Switzerland

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ABSTRACT

The organization of the κ chain constant region gene was compared in DNA from an immunoglobulin-producing mouse myeloma (MOPC 173) and from liver. In situ hybridization using the Southern blotting technique revealed constant region gene-containing EcoRI-DNA fragments of 14 and 20 kb in the myeloma tissue whereas one EcoRI-DNA fragment with a length of 15 kb was found in liver DNA. After enrichment by RPC-5 chromatography and preparative electrophoresis the 14 kb fragment from MOPC 173 DNA and the 15 kb fragment from liver DNA were cloned in the bacteriophage λ vector Charon 4A using in vitro packaging. Extensive characterization of the two fragments by restriction endonuclease mapping, in situ hybridization, and electron microscopy (R-loop and heteroduplex) showed that both fragments contain the constant region but no MOPC 173 variable region gene. Both fragments are homologous over a length of 12.5 kb including the constant region but differ from one another starting about 2.7 kb from the 5' end of the constant region gene. This indicates that the 14 kb EcoRI-DNA fragment from the myeloma tissue clearly resulted from somatic DNA rearrangement although it does not seem to carry the MOPC 173 variable region gene. These observations suggest that somatic DNA rearrangement of immunoglobulin light chain genes can involve both homologous chromosomes.

INTRODUCTION

Direct studies on the structure of immunoglobulin genes are expected to clarify the question of the rearrangement of the variable and the constant region genes and the enigma of allelic exclusion. Variable and constant region gene rearrangement has been extensively studied in the case of mouse λ light chains (1-3), but only limited information has appeared so far in the case of mouse κ chains (4-6) which account for over 90 % of mouse light chains. One important and still unsettled issue is whether both of the homologous chromosomes,

or only one of them undergoes some form of DNA transposition involving the immunoglobulin genes. This question is crucial for an understanding of the molecular basis of allelic exclusion.

Since the first isolation of mRNA for immunoglobulin chains (7) and the demonstration that synthetic genes, made in vitro from mRNA, could be cloned in bacterial vectors (8), it has become evident that such a direct approach, at the DNA level, would be crucial for an understanding of some essential aspects of the immune response. We have therefore cloned immunoglobulin genes from cellular DNA in bacteriophage λ and have compared the organization of the κ chain constant region gene in an immunoglobulin producing mouse myeloma tumor and in liver, which, with respect to immunoglobulin synthesis, can be considered as undifferentiated tissue. We have chosen myeloma MOPC 173, for which the entire amino acid sequence of the light and the heavy chain is known (9), and whose light chain belongs to a different κ subgroup than those under study in other laboratories (4-6).

MATERIALS AND METHODS

Materials. E. coli K802 (hsr^-, hsm^+, $galK^-$, suII lac Y^-, met^-) and the EK-2 λ vector Charon 4A were obtained from F. Blattner (10). DP50 supF was from P. Leder (11). The lysogenic strains BHB2688 and BHB2690 used for the preparation of in vitro packaging extracts were from B. Hohn (12). α^{32}P-dATP and α^{32}P-dCTP were purchased from Amersham or NEN, T4 ligase from Miles, BD-cellulose from Serva; Salmon sperm DNA (Sigma) was extracted three times with phenol before use. Mouse plasmacytoma tumors, provided originally by M. Potter (N.I.H., USA), were maintained by subcutaneous transplantation in inbred Balb/c mice.

Fractionation of mouse DNA. 15 to 25 mg of high molecular weight DNA from Balb/c mouse liver and MOPC 173 myeloma tumor prepared according to ref. 13 was digested to completion with EcoRI and dialyzed against RPC-5 equilibration buffer (1.25 M sodium acetate, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The DNA fragments were then fractionated on a 1.4 x 70 cm RPC-5

column (14-16) using a 4 l linear gradient from 1.45 to 1.85 M sodium acetate. Fractions of 12 ml were collected. In order to detect fragments that contain immunoglobulin gene sequences aliquots of 150 - 400 µl were either directly applied to nitrocellulose filters (BA 85, Schleicher and Schüll) according to ref. 17 or after electrophoretic separation transferred to nitrocellulose filters by the technique of Southern (18) and hybridized with the nick-translated HhaIA fragment of plasmid K38.

Appropriate fractions from the RPC-5 chromatography were pooled, concentrated by ethanol precipitation; the DNA was fractionated on an automated, cylindrical preparative electrophoresis machine. This system which was obtained from B. Richards and J. Pardon at Searle Company (High Wycombe, England) was designed by E.M. Southern (manuscript in preparation) and is now available from Birchover Instruments Ltd. (Letchworth, England). A 0.6 % agarose gel in 40 mM Tris-acetate, 20 mM sodium acetate, 1 mM EDTA, pH 7.2, was used with 2 V/cm. Fractions of about 26 ml were collected every 30 min over a period of 3 days. Fractions containing immunoglobulin gene sequences were identified by hybridization as described above using 2 - 3 ml aliquots and concentrated by ethanol precipitation. Slight impurities in the pooled electrophoresis fractions which inhibited the ligase reaction were removed according to ref. 19 but using BD cellulose instead of BND cellulose. After ethanol precipitation the DNA was dissolved in 10 mM Tris, 1 mM EDTA, pH 8.0, and its concentration was determined by mixing an aliquot with ethidium bromide and measuring fluorescence in a Perkin Elmer fluorescence photometer calibrated with DNA solutions of known concentrations.

Isolation of recombinant phages. A mixture of 0.8 µg of Charon 4A vector "arms" (10), isolated from EcoRI digests by preparative electrophoresis, and 0.4 µg of purified mouse DNA was incubated at 70° C for 5 min, annealed in ice for 30 min, and then ligated in 12 µl of 66 mM Tris, pH 7.5, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM EDTA, 40 mM NaCl, 0.1 mM ATP with 4 units/ml of T4 ligase for 24 h at 10° C. Ligation was

monitored on 0.3 % agarose gels. Subsequently 6 µl of this mixture were used for the "in vitro packaging" reaction according to ref. 12. Phages were then plated on E. coli K802 at about 1000 - 3000 plaques per petri dish and screened using the plaque hybridization assay (20) with the HhaIA fragment as a probe. Plaques that hybridized were replated twice for purification. Bulk production of the recombinant phages was carried out by a plate lysate method (21) using E. coli DP50 supF as a host.

All experiments involving isolation and propagation of recombinant phages were carried out under L3/B2 conditions in accordance with the German guidelines.

Electron microscopic analysis of DNA fragments. EcoRI fragments purified from recombinant phage by agarose gel electrophoresis were hybridized at 10 µg/ml without denaturation under conditions of R-loop formation (22) in the presence of MOPC 173 mRNA (10 µg/ml) purified up to the second sucrose gradient step (23). For heteroduplex analysis, DNA from each of the two fragments (8 µg/ml each) was denaturated and reannealed as described (22) for 4 h. At that point light chain mRNA from A PC 41 (8 µg/ml) was added and the sample was further incubated for 4 h at 53° C. Following spreading of an aliquot (in 50 % formamide and 50 µg/ml of cytochrome C) and shadowing, the grids were examined in a Hitachi electron microscope. The contour lengths were measured on projected photographs with a Numonics digitizer. We thank Dr. J.C. Piffaretti for his help and advice in taking these pictures.

RESULTS

Analysis of mouse restriction fragments containing immunoglobulin sequences. cDNA derived from MOPC 173 κ chain mRNA had been cloned to yield the hybrid plasmid K38 (24). The HhaIA fragment of this plasmid contains the about 850 bp cDNA, while the fragment between the HpaI site and the right HhaI site comprises the constant region gene of the retrotranscript (Fig. 1). In order to identify fragments containing immunoglobulin gene sequences in EcoRI digestion products of DNA from mouse liver

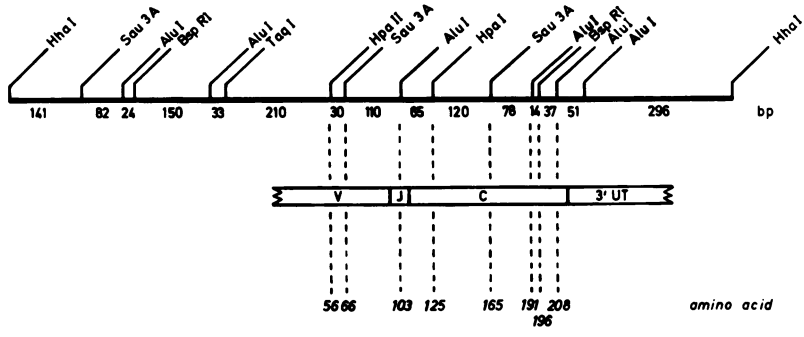

Figure 1. Location of restriction nuclease cleavage sites in the HhaIA fragment of the cDNA-containing plasmid K38. Plasmid K38 (24) was cleaved with HhaI and the largest fragment with a length of 1441 bp isolated. The location of a number of restriction nuclease cleavage sites in the largest HhaI fragment (HhaIA) was determined and compared to theoretical nucleotide sequences derived from the known amino acid sequence of the MOPC 173 κ chain (9). The location of the cloned cDNA is indicated schematically. Distances between restriction sites are given in bp; amino acids are designated by numbers. The positions of the restriction nuclease cleavage sites in the constant region gene and the 3' untranslated region (3'UT) are in accordance with the nucleotide sequence of the MOPC 21 κ chain cDNA (25). A further cleavage site for AluI, however, 8 bp to the right of the AluI cleavage site in the 3'UT region is found in the nucleotide sequence.

and MOPC 173 plasmacytoma cells, the restriction fragments were fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters using the Southern blotting technique (18). As a hybridization probe we used the nick-translated HpaI-HhaI constant region fragment of plasmid K38. The hybridization revealed only one fragment of about 15 kb in liver DNA whereas two fragments of about 14 and 20 kb were observed in the DNA from the MOPC 173 plasmacytoma cells (Fig. 2).

Instead of one fragment containing the constant region gene in the liver DNA, two fragments were thus found in the DNA of the immunoglobulin-producing cells.

When the hybridization was carried out with a probe containing the constant region gene as well as part of the variable region gene specific for MOPC 173 κ chain [HhaIA fragment from plasmid K38 (Fig. 1)] in addition to the fragments found with

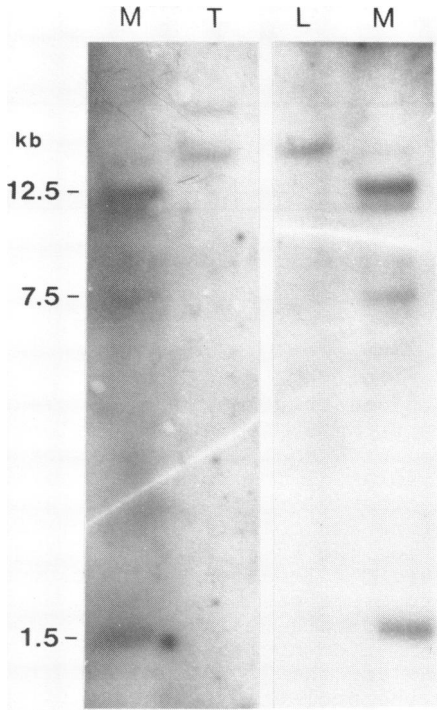

Figure 2. Identification of DNA fragments containing constant region gene sequences in EcoRI cleaved mouse DNA from MOPC 173 and liver. 5 µg each of liver and tumor DNA were cleaved with EcoRI to completion and fractionated on a 0.5 % agarose gel in Tris-EDTA-phosphate buffer (26) at 2 V/cm. After the run the DNA fragments were transferred to a nitrocellulose filter according to Southern (18) including a 5 min exposure of the gel to UV light to introduce breaks into the larger DNA fragments. The filter was then baked for 2 h at 80° C in vacuo and washed before the hybridization in 3 x SSC for 30 min at 68°, subsequently in 3 x SSC, 10 x Denhardt's solution (27) for 3 h and finally washed in 2 x SSC, 6.6 x Denhardt's solution, 50 µg/ml sheared and denatured salmon sperm DNA, 10 µg/ml poly(A), 10 µg/ml poly(C), 0.1 % SDS for 1 h at 70° C (28). For hybridization the latter solution was renewed and supplemented with 10 ng/ml of the constant region gene-containing HpaI-HhaI fragment (Fig. 1) which had been labeled with α^{32}P-dATP and α^{32}P-dCTP to a specific activity of 3 x 10^8 cpm/µg by nick-translation (29,30). After hybridization for 18 h at 70° C the filter was washed six times for 5 - 10 min each in 2 x SSC, 6.6 x Denhardt's solution, 0.1 % SDS at the same temperature and then twice for 15 min each in 0.1 x SSC, 0.1 % SDS at 65° C. The filter was dried and exposed to Kodak X-Omat R film for 9 days with a SE6 intensifying screen from Cawo (Schrobenhausen, West Germany). The slots contained the following: M, marker fragments; T, L, EcoRI fragments of MOPC 173 and mouse liver DNA, respectively.

the constant region probe alone a series (about 10) of smaller DNA fragments were also seen. These fragments gave comparatively weak hybridization signals probably because they contain variable region gene sequences which are related but not exactly homologous to the MOPC 173 variable region gene. Both the MOPC 173 DNA and the liver DNA gave a pattern of weak hybridization to multiple bands. When DNA from 11 - 13 day-old mouse embryos was analyzed for immunoglobulin fragments using the HhaIA fragment as a probe we found exactly the same bands as observed in the liver DNA, which was used in the cloning experiments.

Enrichment for DNA fragments containing immunoglobulin gene sequences. Our protocol consisted in fractionation of mouse EcoRI-DNA fragments by RPC-5 column chromatography (14,15) and subsequent preparative electrophoresis. A similar procedure had been used before in experiments with globin DNA fragments (31) and immunoglobulin variable region DNA fragments (32) from mouse DNA. The two fragments of 14 and 20 kb length of the MOPC 173 DNA hybridizing to the constant region probe (Fig. 2) were separated on the RPC-5 column after the bulk of the DNA had been eluted. The 15 kb fragment from the mouse liver DNA eluted from the column at about the same position as the 14 kb fragment from the tumor DNA. Fractions corresponding to the two latter fragments which are the subject of this study were then pooled and the DNA fragments were further enriched using a preparative electrophoresis system with a discontinuous elution device (E.M. Southern, manuscript in preparation). According to the DNA absorption profiles the fragments were enriched by the RPC-5 fractionation step about 10-fold. Electrophoresis gave another 20-fold enrichment as determined from the amount of DNA in the pools.

Isolation of recombinant phages containing immunoglobulin gene sequences. For the cloning of the enriched EcoRI fragments from mouse DNA the λ phage Charon 4A was used as a vector. Purified vector arms were mixed with the enriched DNA, ligated and phages were produced by in vitro packaging. The yield of plaque forming units (pfu) after ligation and in vitro packaging was about 10^5 pfu per μg of DNA in the ligation mixture, com-

pared to about 10^7 pfu per µg when unrestricted Charon 4A DNA was packaged in vitro. The screening was carried out by the plaque hybridization assay described by Benton and Davis (20) using the HhaIA fragment as a probe. Out of 8000 plaques one positive was found in case of the 14 kb tumor fragment and five positive clones were detected in 35 000 plaques in case of the 15 kb liver fragment. Three of the five positive recombinant phages from the liver DNA cloning experiment were propagated and found to contain the same DNA fragment, although in both possible orientations within the vector phage.

Characterization of the clones containing immunoglobulin gene sequences. In order to localize the constant region gene within the two cloned fragments from liver and MOPC 173 DNA and to identify possible differences between them they were excised from the recombinant phage DNAs by EcoRI digestion and isolated by agarose gel electrophoresis. Cleavage of the two fragments with a number of restriction nucleases revealed that the two fragments are mostly alike and differ from one another only in their extreme 5' portion (Fig. 3). A length of 13.7 kb was found for the fragment isolated from the MOPC 173 DNA whereas a length of 14.9 kb was determined for the liver DNA fragment. For hybridization restriction fragments from both cloned fragments were then transferred to nitrocellulose filter. The data using the HhaIA fragment from K38 (variable and constant region) as a hybridization probe localize immunoglobulin sequences in two parts on both fragments which are indicated by wavy lines. When the probe specific for the constant region gene (HpaI-HhaI fragment, Fig. 1) was used, hybridization occurred only to the right of the HpaI cleavage site, thereby localizing the constant region gene. The position of the HpaI site which correspond to amino acid 125 in the constant region furthermore unequivocally determined the 5'-3' direction of the constant region gene. The hybridization of the HhaIA fragment to restriction fragments from the 5' portion of 173/1 and L/1 is always weaker than to the restriction fragments containing the constant region gene.

Analysis of cloned fragments by electron microscopy. The EcoRI fragments from myeloma DNA (173/1) and from liver DNA

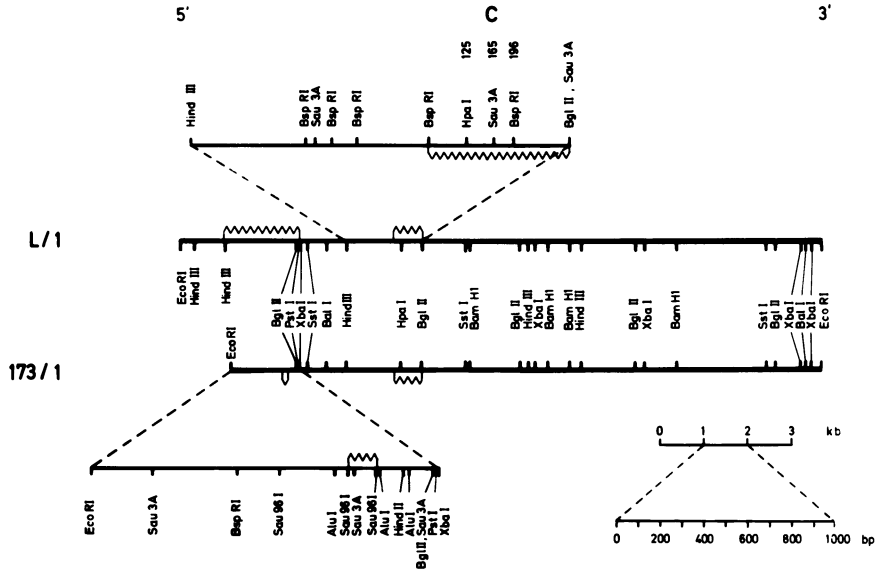

Figure 3. Location of restriction nuclease cleavage sites and immunoglobulin gene sequences in the two cloned DNA fragments from MOPC 173 and mouse liver. Cloned DNA fragments were isolated from the phage DNAs by digestion with either EcoRI alone or EcoRI plus XbaI and preparative agarose gel electrophoresis. The fragments were mapped by a combination of double digestions and partial digestions using λ EcoRI (33) and λdv1 Bsu-DNA fragments (34) as calibration markers. Fragments that hybridize to the HhaIA fragment from K38 are indicated by wavy lines. The location of the constant region gene and the 5'-3' orientation of its noncoding strand are given together with the numbers of some amino acids corresponding to certain restriction nuclease cleavage sites in the constant region. 173/1 denotes the EcoRI fragment isolated from MOPC 173 DNA, L/1 that from mouse liver DNA.

(L/1) were each hybridized under conditions of R-loop formation with light chain mRNA from either MOPC 173 or MOPC 41 myeloma. With both EcoRI fragments, an R-loop was seen at the same distance of one end of the fragment. With both DNAs, in addition to the well identified R-loop structure corresponding to the constant region gene, a non-hybridized tail of 173 mRNA was seen, with the same orientation (Fig. 4). The same pictures were obtained with an mRNA from another κ group, MOPC 41. The unhybridized variable region confirmed the orientation of the

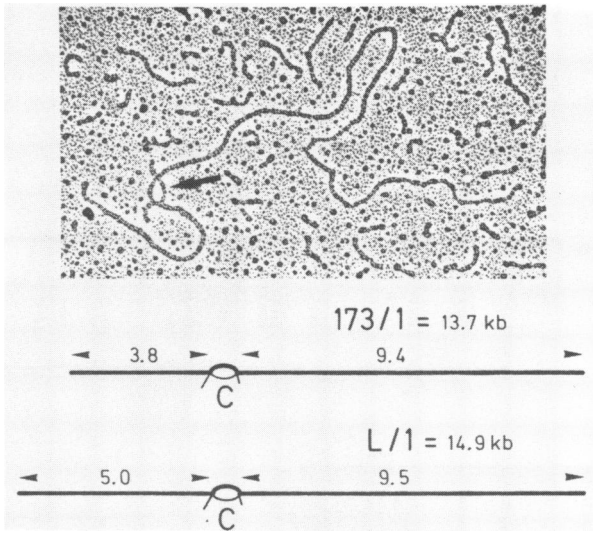

Figure 4. Top: Electron micrograph of the cloned and purified EcoRI fragment 173/1 hybridized under condition of R-loop formation with light chain mRNA from MOPC 173 myeloma (see methods). The R-loop structure (arrow) resulting from the displacement of the constant region gene (plus 3' untranslated) by the mRNA is flanked by the unhybridized poly A tail and by the variable region sequence of the mRNA about 200 and 350 nucleotides respectively. Bottom: Schematic representation of the position of the constant region gene R-loop on either the liver 13.7 kb (L/1) or the 173 14.9 kb (173/1) EcoRI fragments. The measurements result from the scanning of more than 12 individual molecules in each case and the average value is indicated in kb.

constant region gene on these fragments and also suggested that neither of these two DNAs contained the MOPC 173 variable region gene. Under the hybridization conditions used, no pairing of the mRNA to additional segments, such as to J region coding sequences were seen.

To study the sequence organization and the possible relatedness of the two fragments, we performed a heteroduplex analysis of the liver (L/1) and the myeloma (173/1) fragments. That experiment, even though it requires complete denaturation, was performed with the purified EcoRI fragments, rather than with the entire bacteriophage DNA. This was possible because of the absence of single-strand nicks following endonuclease digestion.

Once the heteroduplex was formed, mRNA was added under condition of R-loop formation so that the position of the constant region gene could be easily visualized. An example of the structure observed is shown in Fig. 5. An extensive area of homologous double-stranded DNA is observed over the entire 3' portion of both DNA fragments, and extending about 2.7 kb to the 5' side of the constant region gene. At that point, two single strand tails are seen, indicative of the DNA rearrangement undergone in MOPC 173 DNA compared with the liver DNA fragment. This picture not only demonstrates the somatic rearrangement, but also defines its exact position with respect to the constant region gene.

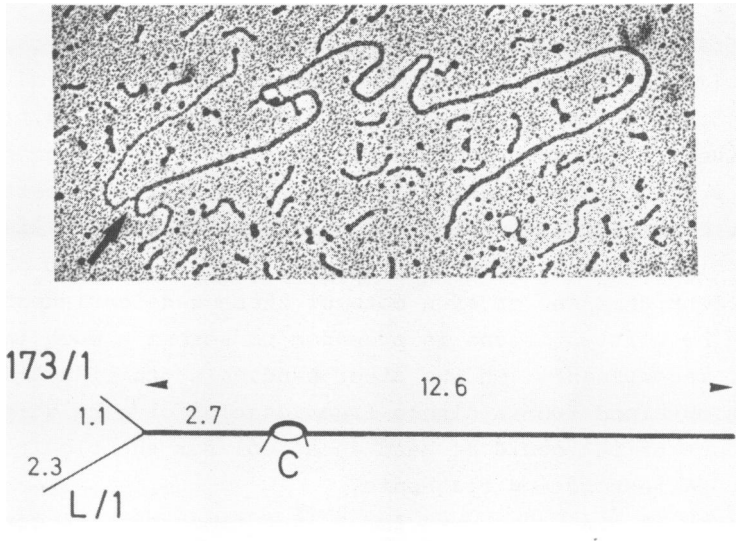

Figure 5. Top: Electron micrograph of a heteroduplex formed between the purified cloned EcoRI fragments from liver (13.7 kb) and from myeloma 173 (14.9 kb). Following annealing of the two fragments, mRNA (MOPC 173) was added under condition of R-loop formation to localize the constant region gene. The molecules obtained consist of thick double-stranded DNA over most of the length of the fragments and of distinct single-stranded extensions of unequal length (arrow). Bottom: Schematic representation of the length and position of the regions of the two fragments (liver and 173) which have either homologous or different sequences. The numbers are as in Fig. 4. The beginning of sequence divergence (single-stranded tails) identifies the position of DNA transposition (see text) with respect to the position of the constant region gene.

Hybridization of mouse DNA restriction fragments to the 5' part of the cloned MOPC 173 DNA fragment. To test whether sequences found in the 5' part of the cloned fragment from MOPC 173 DNA are present only once or are repeated in the mouse genome we isolated the 1.6 kb EcoRI-XbaI-DNA fragment from 173/1 (Fig. 3) and used it as a hybridization probe. Fig. 6 shows that multiple bands were found upon hybridization to DNA fragments from MOPC 173 obtained with a number of restriction nucleases. Multiple bands were also found when mouse liver DNA was analyzed (Fig. 6) indicating that homologous or related sequences to those at the 5' part of the MOPC 173 DNA fragment are present in the mouse genome.

DISCUSSION

The fractionation of DNA fragments by reverse-phase chromatography (14,15), as suggested by others (16), achieves a significant enrichment of specific DNA sequences. In addition, preparative electrophoresis resulted in a further enrichment based on fragment size. The use of these two steps for partial purification explains why it was possible to identify κ light chain specific clones among less than 10 000 recombinant phages. It is evident that one or even both of these gene enrichment steps can be omitted if one is prepared to screen a much larger number of recombinants. On the other hand, a stock of RPC-5 fractions obtained from a single fractionation of a relatively large amount of DNA could be used as a pool for the cloning of other DNA restriction fragments.

Although it has been known for a long time that the constant and the variable regions of immunoglobulin genes are coded for by distinct genes (35), the study of the somatic rearrangement of these variable and constant region genes has only begun recently (1-6). Studies of κ chain genes in myeloma by electrophoresis and blotting of restricted DNA have so far indicated a persistence of the "germ line" constant region gene-containing EcoRI fragment in myeloma DNA (5,6). This has been interpreted as an evidence that somatic DNA transposition, or rearrangement, involved only the immunoglobulin genes on one of the two homologous chromosomes.

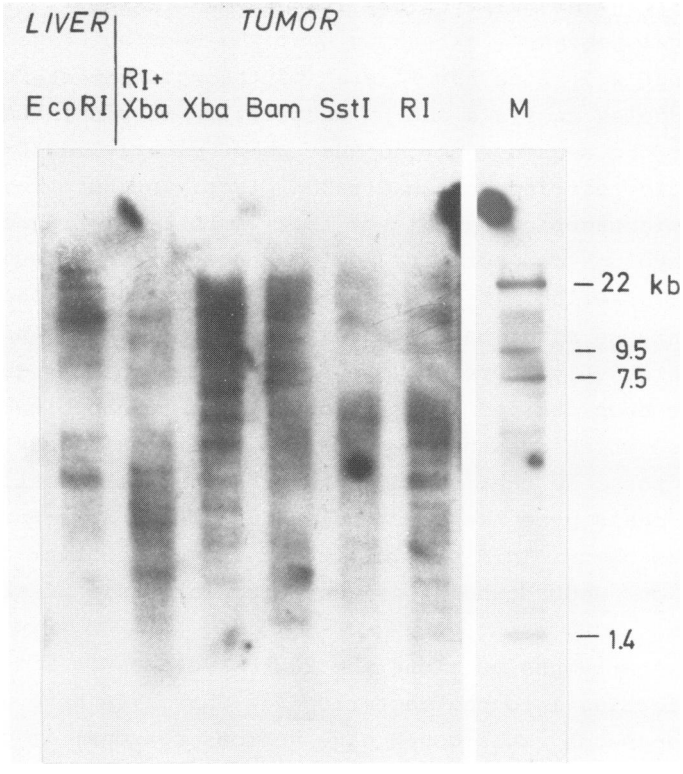

Figure 6. Hybridization of mouse DNA restriction fragments to the 1.6 kb EcoRI-XbaI-DNA fragment from 173/1. 5 µg each of MOPC 173 DNA or mouse liver DNA were cleaved to completion with the restriction nucleases indicated in the figure, fractionated by electrophoresis on a 0.5 % agarose gel and transferred to a nitrocellulose filter (18). Hybridization was with 20 µg/ml of the 1.6 kb EcoRI-XbaI-DNA fragment from 173/1 (Fig. 3) labeled by nick-translation to a specific activity of 10^8 cpm/ng. Procedures were as in Fig. 2 except for the last four washing steps which were in 0.1 x SSC, 0.1 % SDS, at 68° C for 15 min each. Autoradiography was for two days with an intensifying screen. Slot M contained marker fragments and had been exposed for a shorter time.

We have observed by Southern blot analysis that the 15 kb constant region gene-containing EcoRI fragment present in liver and embryo DNA, was no longer seen in DNA from MOPC 173 myeloma. Instead, two EcoRI fragments, respectively 14 kb and approximately 20 kb, were detected with the κ constant region as a probe. Cloning of the myeloma 14 kb fragment and its comparison with

the 15 kb fragment of liver DNA indicated an extensive region of identical sequence, extending over the constant region gene and to about 2.7 kb to the 5' side of the constant region gene. By heteroduplex analysis and by restriction mapping, the comparison of the sequence beyond that point clearly indicated that somatic rearrangement had taken place. Furthermore, the electron microscopic analysis of that 14 kb EcoRI fragment from MOPC 173 myeloma did not give any evidence for the presence of the MOPC 173 variable region gene at the position of DNA rearrangement, as might have been expected from other studies (3-6). Tentatively therefore, we propose that this fragment could have been derived from the one of the two homologous chromosomes which does not express its light chain gene. It is therefore possible that the non-expressed, or allelically excluded, κ chain gene has nevertheless undergone some form of DNA rearrangement. This possibility may have important implications for our understanding of the molecular basis of allelic exclusion.

Several attempts to clone the 20 kb myeloma DNA fragment seen by blotting into the bacteriophage Charon 4A have so far been unsuccessful. This could have various reasons; it could for instance be due to the large size of the myeloma DNA fragment which approaches the maximum theoretical fragment length of 22 kb that can be ligated into the Charon 4A vector phage (10).

Comparison of our physical map of the EcoRI fragment cloned from liver DNA to the one of the EcoRI fragment containing the constant region gene cloned from mouse embryo DNA (5,6) shows that the two fragments are probably identical. The size of the embryonic EcoRI fragment has been determined to be 15 kb (5) and 16 kb (6) which is in good agreement with the length of 14.9 kb found for the liver EcoRI fragment. Moreover the location of the constant region gene (5,6) as well as the positions of some restriction nuclease cleavage sites (6) are alike in the embryonic and the liver EcoRI fragment.

Both of the cloned liver and myeloma EcoRI fragments, when analyzed by Southern blots with a radioactive probe containing part of the variable and the J region of MOPC 173 light chain,

showed a weak but reproducible hybridization to a restricted region on the 5' end of the cloned fragments. In case of the 14 kb myeloma fragment this region has been narrowed to a restriction fragment of approximately 130 bp (Fig. 3). It is not yet clear whether the weak hybridization to the 5' end of the liver DNA is due to the presence of the same fragment. Because both the myeloma and the liver fragment do not contain the MOPC 173 variable region gene as has been shown by electron microscopy we attribute the weak hybridization to one or more J region coding segments with homology to the MOPC 173 J region. Two different J regions, namely the MOPC 321 (5) and the MOPC 41 (6) J region have so far been identified on the embryonic EcoRI fragment approximately 2.8 and 3.7 kb, respectively, from the 5' end of the constant region gene. In this respect it is interesting to mention that the J region of the MOPC 173 κ chain has the same amino acid sequence as the one of the MOPC 41 κ chain and differs from the MOPC 321 J region sequence only in one amino acid (36).

Another interesting, although unexplained, observation is the hybridization of the 5' part of the MOPC 173 14 kb fragment to multiple bands in total mouse DNA restriction fragments by Southern blot analysis. Depending on the exact nature of the probe sequence which was responsible for that hybridization, one could attribute it to cross-hybridizing variable region genes or to sequences flanking a variable region gene (4).

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NOTE ADDED IN PROOF

On the request of a referee we did the following experiment

in order to show directly that the 14 kb EcoRI fragment from myeloma DNA is not a cloning artifact. EcoRI digested DNA from mouse liver and the myeloma MOPC 173 were mixed and electrophoresed in parallel to the cloned 14 kb fragment on a 0.5 % agarose gel. After blotting and hybridization with a labelled probe containing the constant region gene an autoradiograph of the filter showed the three expected bands in the lane containing the mixed mouse DNAs. In addition to the 20 kb fragment from myeloma DNA the 15 kb fragment from liver DNA and the 14 kb fragment from myeloma DNA were clearly separated from one another. The latter had exactly the same mobility as the cloned 14 kb myeloma fragment.

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