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Cloning of V region fragments from mouse liver DNA and localization of repetitive DNA sequences in the vicinity of immunoglobulin gene segments

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ABSTRACT

Two different kappa light chain genes have previously been isolated from one mouse myeloma. The V (variable, abbreviations in ref. 2) gene segments of the two genes were now used to identify their germline counterparts in EcoRI digests of mouse liver DNA. In addition two sets of related V gene segments were found which hybridize with either of the two DNA probes. Five of the V region fragments of one set were cloned in a lambda phage vector and partially characterized by restriction mapping and Southern blot hybridization. Repetitive DNA sequences were found on each of the five fragments as well as on other cloned immunoglobulin gene containing fragments. Cross-hybridization between some but not all of the regions containing repetitive DNA sequences was observed.

INTRODUCTION

One feature of the generation of antibody diversity is a rearrangement of the genomic DNA in the course of which different V and J gene segments are joined (3,4). Rearranged V gene segments and their non-rearranged counterparts have been isolated from mouse myeloma and mouse embryos, respectively (3,4). In hybridization experiments with V gene specific probes it was found that both in committed and in non-committed tissues there exist families of related light chain V gene segments (5-7). Two of the related V gene segments have also been sequenced (8).

In previous work of this laboratory two kappa light chain genes were isolated from a mouse myeloma (9,10) which had been designated myeloma T (10). We now turned to the question whether the two rearranged V gene segments are derived from different families or sets of germline V gene segments. We therefore

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identified, in a non-committed tissue, the counterparts of the two rearranged V gene segments and characterized the respective sets of related V gene segments. As an immunologically non-committed tissue we chose, as in previous work (9-11), mouse liver. In the present report we also describe an interesting feature of several of the immunoglobulin gene containing fragments, that is the occurrence of repetitive sequences in the vicinity of the immunoglobulin gene segments.

MATERIALS AND METHODS

Most materials and methods were as in the preceding papers (10,11). The EcoRI fragments L2-L6 from mouse liver DNA were enriched by RPC-5 chromatography (legends of Fig. 1 of the present paper and of Fig. 4 of ref. 10) and preparative electrophoresis analogously to the procedure described in ref. 10. Cloning in lambda-gtWES (12) was as in ref. 10,11. The probe used for identification of the clones was the V-T1 region subclone containing the rearranged V gene segment of the 14 kb myeloma fragment T1 (Fig. 2b of ref. 10).

RESULTS

Identification and cloning of EcoRI fragments from liver DNA containing kappa V gene segments. DNA fragments from mouse liver DNA containing V gene segments were identified by Southern blot hybridization of EcoRI digested DNA with two different V region probes. The probes were the V-T1 and the V-T2 region subclones, which contain the rearranged V gene segments of the two kappa chain genes present in the DNA of myeloma T (10). Fig. 1 shows that in a two-dimensional Southern blot hybridization experiment (first dimension: RPC-5 chromatography, second dimension: gel electrophoresis) both probes hybridized to a number of different EcoRI fragments. These fragments are parts of two sets of closely related V gene segments. The germline counterparts of the rearranged V gene segments may be expected to give the strongest hybridization signal. An exact quantitation of Southern blots is, of course, impossible and, if there are several strongly hybridizing fragments in a blot, other methods have to be employed to identify the germline V gene segments.

With the V-T1 region probe three strong bands were observed.

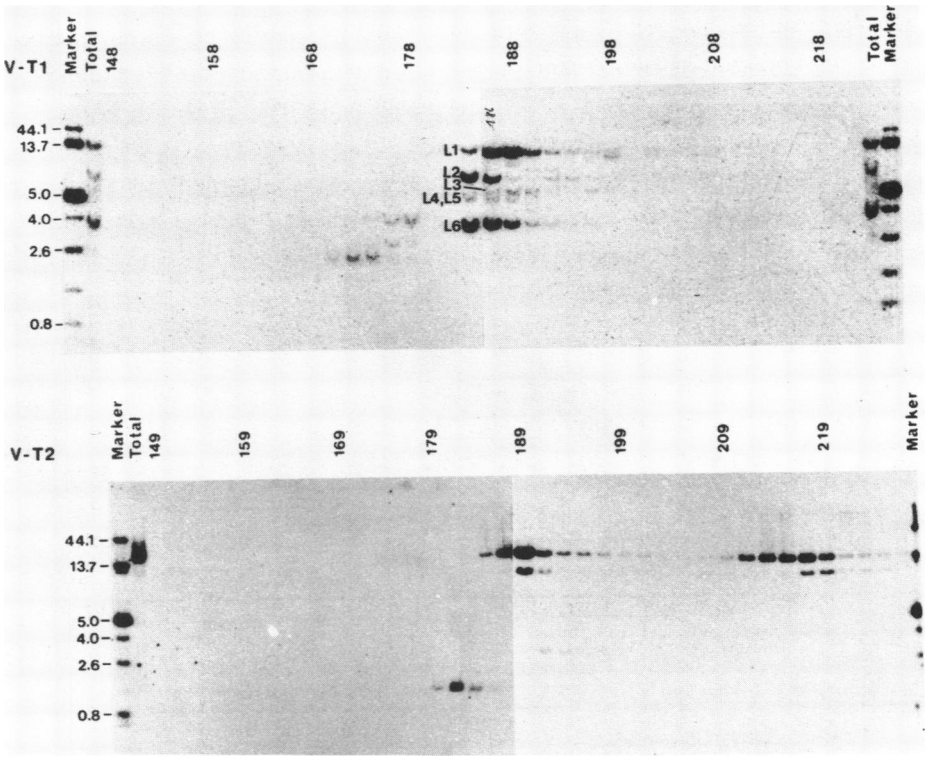

Figure 1. Identification of V gene sequences similar or identical to the V gene segments of the 14 or 20 kb fragments from myeloma T DNA in EcoRI digests of mouse liver DNA. 0.2 ml aliquots of every second fraction from the RPC-5 chromatography run of the DNA digest of Fig. 4 of ref. 11 were analyzed by electrophoresis and Southern blotting using the V-T1 and V-T2 region subclones (Fig. 2b,c of ref. 10) as probes. Fractions of the RPC-5 chromatography are designated by numbers. Fragments L2-L6 which were subsequently cloned are indicated. Hybridization to the 15 kb fragment L1 was due to the presence on the probes of sequences from the intron between the J and C segments (Fig. 2b,c of ref. 10).

One was due to the 15 kb EcoRI fragment L1 from liver DNA containing the 3' flanking sequences of the J region which are also present in the probe (see Fig. 2a and b of ref. 10). The two other strongly hybridizing fragments L2 and L6 and the more weakly hybridizing fragments L3-L5 were cloned in lambda-gtWES following the procedures used also in ref. 10 and 11. According

to restriction nuclease mapping and Southern blot hybridizations with the V-T1 region probe the fragment L6 seems to contain the germline counterpart of the rearranged V gene segment of fragment T1 (compare the map of L6 in Fig. 2 of the present paper with the map of T1, previously named 173/1, in Fig. 3 of ref. 9). On the basis of the same type of experiments fragment L2 does not contain the germline V gene segment; fragments L3-L5 are unlikely candidates because of their weak hybridization with the V-T1 probe.

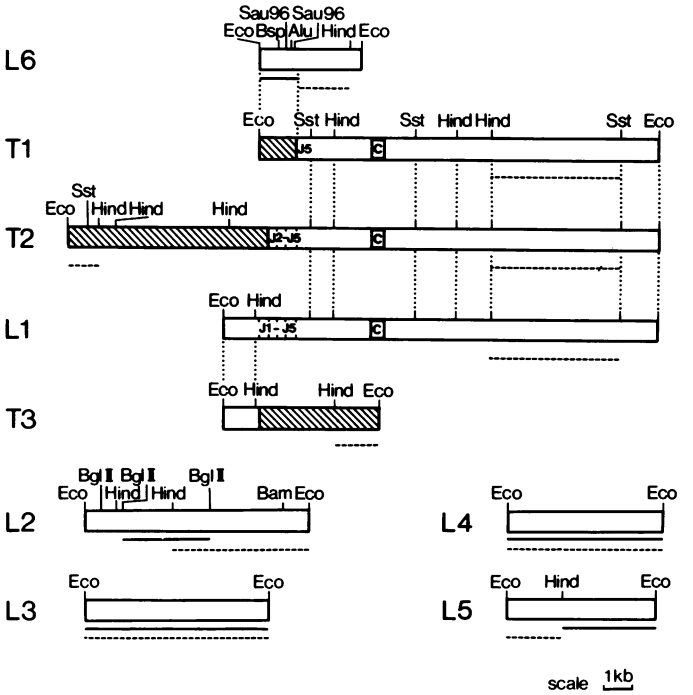

Figure 2. Repetitive DNA in cloned fragments from myeloma T (T1-T3) and liver DNA (L1-L6). Translocated DNA sequences in myeloma DNA are indicated by hatching. Restriction fragments derived from L2-L6 which hybridize to V-T1 in Southern blots are underlined; in L6 the line extends over the region translocated in fragment T1. Repetitive sequences (indicated by broken lines) were identified by Southern blot hybridization with total mouse DNA as a probe as shown for T2 in Fig. 3. Partial restriction maps of T1-T3 and L1 were taken from refs. 9-11, those for L2-L6 were determined by single and double digestions of the corresponding recombinant phage DNAs.

Hybridization with the V-T2 region probe led to the identification of only one strongly hybridizing fragment in addition to fragment L1 and at least three more weakly hybridizing fragments (centred around fractions 181, 189, and 219). The strongly hybridizing fragment was about 14 kb in length and was found to be eluted from the RPC-5 chromatography column in fractions 211-219 (Fig. 1). For identification of the fragments we concentrated aliquots of the RPC-5 fractions 189, 213, and 217 by ethanol precipitation and carried out a second digestion of the DNA fragments with PstI as well as with BamHI. Southern blots of the PstI and BamHI digests were then hybridized with the V-T2 region probe (not shown). Comparison of the hybridization patterns obtained with the restriction maps of L1 and T2 (9,10) confirmed our expectation that the 14 kb fragment (fractions 211-219) comprises the germline equivalent of the rearranged V gene segment of fragment T2 and that the RPC fraction 189 (and its neighbors) contained fragment L1.

Repetitive DNA sequences are found in the vicinity of kappa V and C gene segments. The presence of repetitive DNA sequences on cloned DNA fragments containing immunoglobulin gene segments was noticed first in hybridization experiments of nick-translated recombinant phage DNA with blots of EcoRI digested mouse liver DNA. Ch4A-T2 DNA (2), for instance, hybridized to a spectrum of mouse liver DNA fragments of different sizes. Hybridization, however, did not occur to all EcoRI fragments derived from mouse liver DNA since the hybridization pattern did not coincide with the gel electrophoretic pattern of DNA fragments as observed by ethidium bromide staining. The hybridization was not changed by the presence of an excess of cold Charon 4A DNA as competitor. These experiments indicated that the cloned myeloma fragment T2 contains sequences dispersed throughout the mouse genome at many sites.

In order to locate the repetitive DNA sequences on fragment T2 we carried out the hybridization experiment in the reverse way using nick-translated mouse myeloma DNA and blots of Ch4A-T2 DNA digested with various restriction nucleases (Fig. 3 A and B). Under the hybridization conditions described in the legend to Fig. 3 we expected that only repetitive sequences (more than

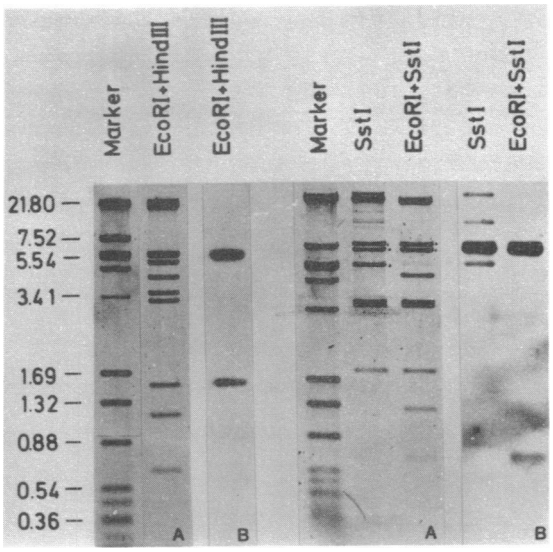

Figure 3. Localization of repetitive DNA sequences on the myeloma fragment T2. (A) 0.5 µg each of Ch4A-T2 DNA were digested with the restriction nucleases indicated and fractionated on a 1 % (HindIII experiment) or a 0.7 % agarose gel (SstI experiment) in the presence of ethidium bromide. Marker fragments were as in Fig. 3 of ref. 10. (B) Autoradiograms of blots of the gels shown in (A) after hybridization with total mouse myeloma DNA (nick-translated to a specific activity of $5 \cdot 10^6$ cpm/µg) at 2 µg/ml. Washing steps and autoradiography were as in Fig. 3 of ref. 10.

about 10^2 copies per genome) would show up in autoradiograms. Only two fragments were identified in an EcoRI + HindIII digest. In the SstI digest a double band (7.5 and 6.8 kb) and a band of 5.9 kb were identified; the two faint bands of high molecular weight can be attributed to partial digestion products. In the SstI + EcoRI digest the double band remained unchanged while the 5.9 kb fragment of the SstI digest gave rise to a 0.7 kb fragment (located at the extreme left of the map of fragment T2, Fig. 2). Two regions comprising repetitive DNA sequences were thus identified on the myeloma fragment T2 as shown in Fig. 2, one about 5.0-6.5 kb upstream of the V gene segment, the other 3.5-8.0 kb downstream of the C gene segment.

As expected, also the myeloma fragment T1 and the liver fragment L1 contained the repetitive DNA region downstream of the C gene segment (Fig. 2). No repetitive DNA sequence has been found under our hybridization conditions upstream of the V gene segment on T1 and upstream of the J gene segments on L1.

Regions containing repetitive DNA sequences have also been found on the DNA fragments L2-L6 cloned from liver DNA which contain V gene segments and/or flanking sequences related to

V-T1 region sequences (Fig. 2). Similarly, on the myeloma fragment T3, known to contain a 3' flanking sequence of a kappa V gene segment (10) repetitive DNA sequences have been found (Fig. 2).

We have used fragment T3 to investigate whether cross-hybridization between some of the repetitive DNA sequences identified above with total mouse DNA does occur. To this aim DNA of the cloned fragments L1-L6, T1 and T2 was cleaved with restriction nucleases and analyzed in Southern blotting experiments with nick-translated fragment T3 (not shown). Most of the fragments resp. regions of fragments containing repetitive DNA sequences did cross-hybridize with fragment T3. Some differences, however, were observed; no cross-hybridization was found to fragments L5 and L6; only parts of the repetitive regions of fragments L1, L2, T1, and T2 which had been identified by hybridization with the total mouse DNA (Fig. 2) hybridized with fragment T3 (experiment not shown). No fragment was found which hybridized to T3 but not to total mouse DNA. The results indicate that some but possibly not all of the repetitive DNA sequences in the vicinity of immunoglobulin gene segments are related to each other.

DISCUSSION

In the first part of the paper multiple EcoRI fragments from the mouse genome are described which comprise sequences homologous to the two rearranged V region segments of myeloma T. These findings are in agreement with similar observations made for other kappa light chain (5-7) and also for heavy chain V region genes (13-15) and contribute to the growing evidence that multiple V gene segments play an important role in the generation of antibody diversity. It is also interesting to note that with the two V region probes two completely different sets of V gene fragments were identified indicating that, in the DNA of myeloma T, V gene segments from two unrelated sets have been rearranged. Whether the two germline V gene segments which have been identified by restriction mapping and hybridization are fully identical with the respective rearranged V gene segments has to be shown by sequence analysis.

In the second part of the paper repetitive DNA sequences are described which were found upstream or downstream of V and downstream of C gene segments. From the observed hybridization of the repetitive DNA sequences to the whole size-spectrum of EcoRI fragments from mouse liver DNA we conclude that the repetitive sequences occur in the mouse genome at many different sites and are not part of mouse satellite DNA sequences which yield completely different patterns on EcoRI digestion (16).

Recently repetitive DNA sequences in the neighborhood of coding segments of DNA have been identified on cloned fragments containing genes for mouse globin (17), human globin (18), rabbit globin (19,20) and chicken conalbumin (21). In view of the potential structural and/or functional significance of the repetitive DNA sequences it is an interesting observation that some but not all of the repetitive DNA sequences near immunoglobulin gene segments cross-hybridize. Determination of the nucleotide sequences of these regions will have to show exactly how related the sequences are. Further experiments are also needed to determine the exact sizes and copy numbers of the sequences in the mouse genome and to decide whether the sequences are transcribed. In this context it is interesting to clarify the relationship of the repeated sequences near the immunoglobulin gene segments to those repeated sequences identified in rapidly reassociating mouse DNA (22) and in cDNA clones prepared from double stranded regions of mouse pre-mRNA (23). A detailed analysis of the repetitive sequences appears to be necessary because they may play a role not only in the organization and expression but possibly also in the rearrangement of the immunoglobulin genes.

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