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A rearranged DNA sequence possibly related to the translocation of immunoglobulin gene segments

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ABSTRACT

A 5.3 kb EcoRI fragment (T3, abbreviations in ref. 2) has been cloned from DNA of a kappa light chain producing mouse myeloma. The fragment hybridizes to the 5' flanking sequences of the J1 gene segment but not to C gene sequences of kappa light chain DNA. Restriction nuclease mapping and partial nucleotide sequencing showed that the fragment consists of sequences from the 5' side of the J1 and from the 3' side of a V gene segment, which apparently had been linked in a genomic rearrangement process. These rearranged flanking sequences are not the flanking sequences of the V and J gene segments which had been joined to form the two kappa light chain genes of the myeloma. Fragments with the hybridization properties of T3 have been found also in two other kappa and one lambda chain producing myelomas. The linking of flanking sequences in the myeloma genome is discussed with respect to the mechanism of recombination between V and J gene segments.

INTRODUCTION

Rearrangement of immunoglobulin light chain gene segments results in the joining of V (variable) and J (joining) gene segments (3,4). Four mechanisms have been proposed for the V-J recombination (5). 1) The copy-insertion model proposes that a V gene segment is duplicated and inserted upstream of a J gene segment. 2) The excision-insertion model assumes that the V gene segment itself is excised and then integrated. 3) According to the inversion model a DNA segment is inverted which results in the joining of a V gene segment to a J gene segment in the correct orientation. 4) The deletion model proposes that the DNA segment separating V and J gene segments in germline DNA is deleted in the course of the V-J joining.

In sequencing studies with cloned V and J gene fragments a characteristic heptanucleotide sequence was found adjacent to

the 3' ends of V gene segments and adjacent to the 5' ends of the J gene segments (6,7). In addition identical hepta- to deca-nucleotide sequences have been detected (6) in both flanking sequences 10-25 nucleotides away from the first one. The repeated sequences have been used in a specific version of the deletion model: they would allow the formation of stem structures which bring V and J gene segments together; sequences in-between the two segments may then be excised in a recombination process which leads to the joining of V and J gene segments (6, 7). The deletion model has also been tested experimentally making use of the fact that the other mechanisms should leave the flanking sequences of the recombining V and J gene segments (in a rearranged form) within the myeloma DNA. However, no DNA fragments which could be attributed to the rearranged flanking sequences were detected in Southern blot hybridization experiments with probes containing flanking sequences of V and J gene segments of a lambda light chain (6). A similar experiment using sequences 5' to the recombining J gene segment of kappa light chains as a probe has not been reported yet, although preliminary evidence has been interpreted to support the deletion model (7).

In this report we describe a specific DNA fragment which must have been generated by a recombination event between sequences 5' to the J1 gene segment and sequences 3' to a V gene segment. This fragment was isolated from the DNA of a kappa light chain producing mouse myeloma which is, as in the preceding paper (8), designated myeloma T. The occurrence of the fragment in DNA of myeloma T and of similar fragments in other mouse myeloma DNAs is discussed with regard to the V-J recombination mechanism.

MATERIALS AND METHODS

Materials and methods were as in the preceding publication (8) except for the following items.

Preparation of mouse DNAs. High molecular weight DNA from Balb/c mice was prepared according to Blin and Stafford (9) and further purified by equilibrium centrifugation in CsCl. Frozen tumors (MOPC321, MOPC41, and RPC20) were gifts of I. Schechter.

DNA of myeloma T and MOPC21a were gifts of G. Raydt. Mouse liver DNA was a gift of R. Summer.

Cloning procedures. The 5.3 kb EcoRI fragment (T3) identified in the DNA of myeloma T with the J region subclone (indicated schematically in Fig. 2a of ref. 8) was cloned in lambda-gtWES (10) (obtained from P. Leder) after enrichment by RPC-5 chromatography and preparative gel electrophoresis analogously to the procedure for cloning the 20 kb myeloma fragment (8). $3 \cdot 10^6$ plaques per µg of purified mouse DNA were obtained after ligation and in vitro packaging. Screening of about 30 000 recombinants with the J region subclone as a probe by the plaque hybridization assay (11) yielded 8 positive clones. Three of these were plaque-purified and propagated using E. coli LE392 (10) as a host. All three clones contained the 5.3 kb EcoRI fragment as well as a second EcoRI fragment which was of different size in individual clones and did not hybridize to our probe. The 5.3 kb fragment was then recloned in pBR322 and a subclone of the 1.15 kb HindIII fragment (T3 subclone, Fig. 2c) was prepared in pBR322, following procedures described in the preceding paper (8).

DNA sequencing. Restriction nuclease fragments were isolated from polyacrylamide gels by elution with 1 M NaCl and filtering through Whatman 3MM cellulose paper (W. Fiers, personal communication), labeled at their 5' ends with ^{32}P and cleaved at internal restriction sites. Single-end-labeled fragments were then isolated and sequence analysis was performed by the chemical degradation method of Maxam and Gilbert (12 and personal communication). A purine specific cleavage reaction was employed as described in (13). 8 % and 20 % sequencing gels were used as described (14) with the buffer system of (15 and personal communication). 8 % gels were autoradiographed without screens, for 20 % gels intensifying screens (Ampli Universal) were used.

RESULTS

Identification of an unexpected 5.3 kb EcoRI fragment (T3) in myeloma DNA hybridizing to a J region probe. DNA of the mouse myeloma T and of mouse liver was digested to completion with EcoRI and analyzed by the Southern hybridization technique

(16) with the J region subclone (Fig. 2a of ref. 8). Fig. 1 shows that this subclone hybridized to the 15 kb EcoRI fragment (L1) from liver DNA from which it was derived (8) and to the 14 and 20 kb DNA fragments of myeloma T (T1 and T2). This was expected because by the V-J recombination events leading to the two myeloma fragments not all of the sequences present in the J region subclone are removed (8). In addition, however, a 5.3 kb EcoRI fragment of the myeloma DNA digest was found to hybridize to the probe. It was designated fragment T3. Since this fragment did not hybridize to the C region subclone (Fig. 1 of ref. 8; see also Fig. 5 of the present paper) and it was also not found in EcoRI digested liver DNA another rearrangement of J region sequences must have occurred in addition to the known V-J recombination event (3,6). We therefore decided to clone this fragment in order to study its sequence organization and eventually define the type of recombination by which this fragment has been generated.

Sequence organization of the T3 fragment. The T3 fragment was cloned into lambda-gtWES after enrichment by RPC-5 chroma-

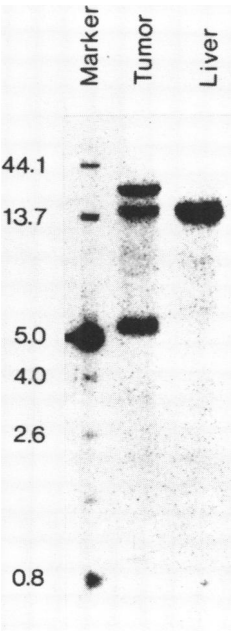

Figure 1. Identification of the rearranged fragment T3 by filter hybridization of EcoRI digested DNA from mouse liver and myeloma T with the J region probe (Fig. 2a of ref. 8). Hybridization marker, myeloma and liver DNA fragments were fractionated on a 0.5 % agarose gel, transferred to a nitrocellulose filter and hybridized as described in the legend to Fig. 1 of the preceding paper (8).

tography and preparative electrophoresis. It was then recloned in pBR322 and preparative amounts were isolated by gel electrophoresis to construct a physical map (Fig. 2) by restriction nuclease digestion and Southern blot hybridization with the J region probe. In this analysis it became clear that the left

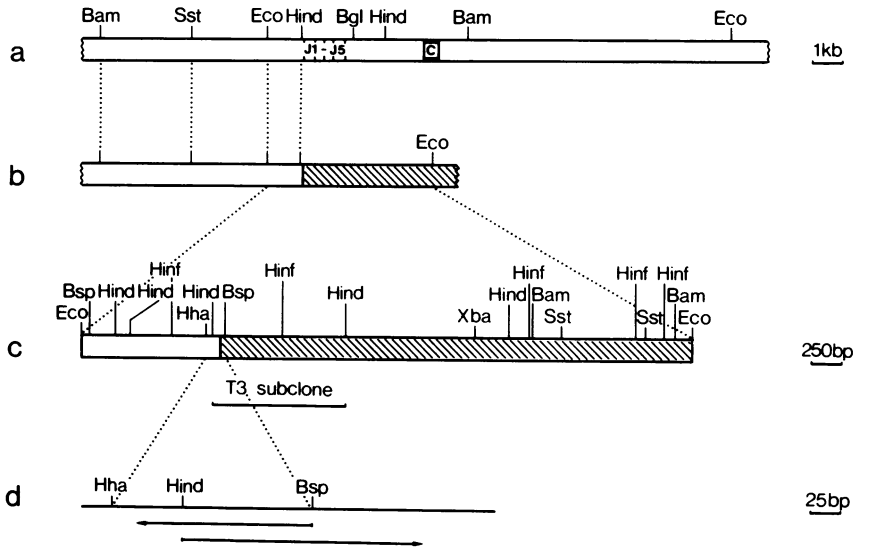

Figure 2. Restriction maps of the rearranged fragment T3 and related DNA. (a), partial restriction map of about 22 kb of DNA surrounding the J and C gene segments in mouse liver DNA (taken from ref. 8). (b), partial restriction map of about 11 kb of myeloma DNA comprising the 5.3 kb EcoRI fragment T3 (expanded in panel c of this figure, see below). This map was obtained by EcoRI (Fig. 1), BamHI and SstI (not shown) digestions of DNA of myeloma T and Southern blot hybridization using the J region subclone (Fig. 2a of ref. 8) as a probe. Those fragments which were specific for myeloma DNA (not found in liver DNA digests) and which were not derived from either of the two rearranged kappa chain genes present in this myeloma are shown. The map of the cloned T3 fragment (panel c) was used for the construction of this map. (c), restriction map of the cloned 5.3 kb EcoRI fragment T3 from myeloma DNA. Southern blot hybridizations with the J region probe were used to identify fragments containing the rearranged 5' flanking sequences of the J gene cluster. For BspRI and HinfI only the relevant sites are shown. No cleavage sites were found for BglII, HpaI, HpaII, and PstI. The 1.15 kb HindIII fragment subcloned in pBR322 (T3 subclone) is indicated. DNA segments which were found to be translocated in myeloma DNA (Fig. 3 and text) with respect to the sequence organization in liver DNA are indicated by hatching. (d), sequencing strategy of the region comprising the site of recombination on T3. Horizontal arrows indicate the directions and extensions of sequence determination. For sequencing the 1.15 kb HindIII fragment and the 1.15 kb BspRI fragment were isolated, labeled and cleaved with HinfI and HhaI, respectively. The scale for (a) and (b) is in kb, that for (c) and (d) in bp.

part of T3 as shown in Fig. 2c corresponds to the region between the left EcoRI and HindIII sites of liver DNA sequences (Fig. 2a) (which are part of the 15 kb liver fragment L1). About 100 bp to the right of the HindIII site a BspRI site is found in T3 which is absent in the liver DNA sequence (and also in embryo DNA sequences, ref. 6,7). This confines in T3 the region of recombination to the short segment of DNA between the HindIII and BspRI sites.

According to restriction nuclease digestion experiments the homology between liver and myeloma DNA extends beyond the left EcoRI site in Fig. 2a and b. Mapping of SstI and BamHI fragments in myeloma DNA by hybridization with the J region probe led to the identification of two restriction sites at similar if not identical positions as in liver DNA (Fig. 2a and b).

In order to identify precisely the site of recombination on the T3 fragment we sequenced the region between the HindIII and BspRI sites indicated in Fig. 2d. The sequence is presented in Fig. 3 and compared to the 5' flanking sequence of J1 (6) and 3' flanking sequences of kappa V gene segments from embryo DNA (6,7). The left part of the DNA sequence of T3 is identical to the one flanking the J1 region up to the heptanucleotide sequence $\begin{matrix}5'\ CACTGTG\ 3'\\ 3'\ GTGACAC\ 5'\end{matrix}$; the homology ends one base pair before the J1 sequence starts in the J region clone. The following T3 sequence is rather homologous to the known 3' flanking sequences of kappa V gene segments (6,7). It is particularly striking that the heptanucleotide is followed by its inverted repeat, that is by $\begin{matrix}5'\ CACAGTG\ 3'\\ 3'\ GTGTCAC\ 5'\end{matrix}$. Among the next 20 nucleotides 10-15 are identical in the T3 and the V kappa sequences (if an one nucleotide gap is inserted into the VK21-C sequence). The heptanucleotide and the other blocks of conserved V kappa sequences have been assumed to be involved in the joining of V and J gene segments because they are also found, in the inverted order, on the 5' side of all kappa J gene segments, so that stem structures could be formed (6,7). We therefore conclude that the T3 sequence has been generated in myeloma DNA by a recombination event (or a series of events) which links the heptanucleotide at the 5' side of J1 to the one at the 3' side of a V gene seg-

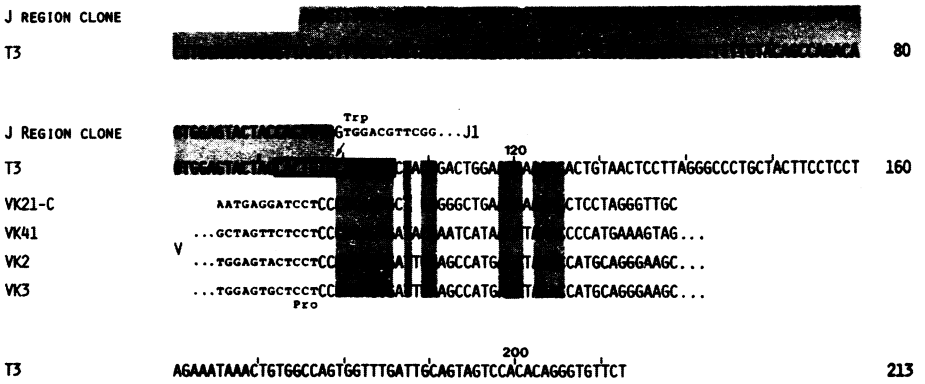

Figure 3. Nucleotide sequences of the junctional region of the rearranged fragment T3 and its comparison to the 5' flanking sequence of the J gene cluster from embryo DNA and to the 3' flanking sequence of kappa chain V gene segments. The sequence of the J region clone was taken from (6); it differs from the one of ref. 7 in two nucleotides. The VK21-C sequence was from (6); VK41, VK2, and VK3 from (7). Sequences identical between the J region clone and T3, and between T3, VK21-C, VK41, VK2 and VK3 are shadowed. Note that an one nucleotide gap has been inserted into the VK21-C sequence to obtain the best homologous pairing. The two heptanucleotide sequences (6,7) flanking the site of recombination on the T3 fragment (arrow) have been boxed. Large and small capital letters represent flanking and coding sequences, respectively. The codon for Pro(95) of the V gene segments and the one for Trp(96) of the J1 gene segment are indicated.

ment.

It is unlikely that fragment T3 has been changed during cloning and propagation of the clone in E. coli: in Southern blot hybridizations with the J region probe the 1.1 kb BspRI fragment containing the recombination site of T3 (Fig. 2c) from cloned DNA was seen to comigrate with the corresponding fragment from uncloned DNA.

Fragment T3 is not directly derived from the V-J recombination events leading to the formation of the kappa chain genes of myeloma T. The kappa chain genes in the fragments T1 and T2 of myeloma T have been formed by joining V gene segments to the gene segments J5 and J2, respectively (8,17). Since fragment T3 contains the 5' flanking sequence of J1 it was not, or at least

not directly, formed in the V-J joining process which led to the formation of T1 and T2.

We next asked the question whether the 3' flanking sequence of a V gene segment present on T3 is identical to one of the flanking sequences of the two germline V gene segments which had been rearranged to form the two kappa chain genes present in myeloma T. The two EcoRI fragments comprising these V gene segments have been identified in liver DNA (18). The V gene segment that has been rearranged to form the 14 kb EcoRI fragment (T1) was found on a 3.5 kb EcoRI fragment in liver DNA which has been cloned and characterized (L6 in ref. 19). No hybridization in Southern blotting experiments (not shown) was found between L6 and the nick-translated T3 subclone (Fig. 2c) which contains part of the 3' flanking sequence on T3. The two sequences are therefore clearly different. The V gene segment which has been rearranged to form the 20 kb myeloma fragment (T2) is present on a 14 kb EcoRI fragment in liver DNA; this fragment was found in fractions 211-219 of an RPC-5 chromatography run of EcoRI digested liver DNA (Fig. 1 of ref. 19). When the RPC-5 fractions were analyzed with the T3 subclone in order to identify the EcoRI fragment that contains the V flanking sequence present in T3, one fragment was found which gave rise to the strongest hybridization signal (Fig. 4) and was therefore probably the one we were looking for. This fragment was also about 14 kb in length but appeared in fractions 201-209 and is therefore different from the 14 kb fragment detected with the V region subclones (compare Fig. 4 of the present paper with Fig. 1 of ref. 19). It is concluded that the 3' flanking sequence on the T3 fragment is different from the ones of the germline equivalents of the V region segments of T1 and T2.

Fragments with the hybridization properties of T3 are found also in other myeloma DNAs. In order to find out whether similar rearrangements had occurred also in other myeloma DNAs Southern blot hybridization experiments were performed with EcoRI digests of DNA from three other kappa chain producing myelomas (MOPC21a, MOPC41, MOPC321) and one lambda chain producer (RPC20) using both C and J region subclones as probes (Fig. 5).

With the C region probe the known EcoRI fragments containing

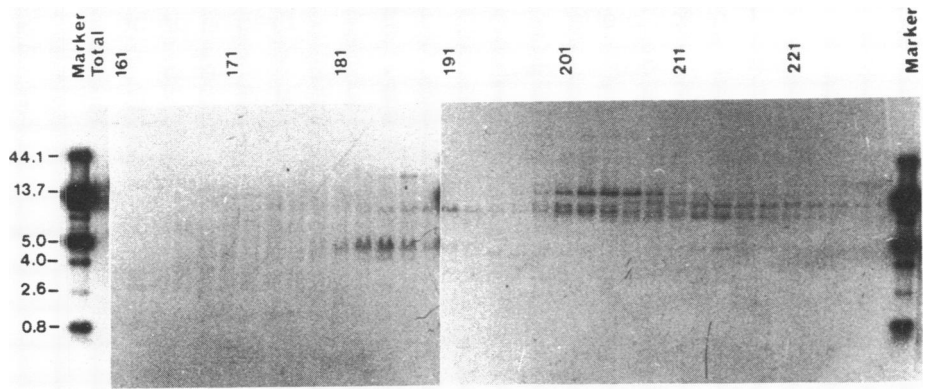

Figure 4. Analysis of EcoRI fragments from mouse liver DNA that hybridize to the T3 subclone. 0.2 ml aliquots of every second fraction of an RPC-5 chromatography run (20 mg of DNA, 80 fractions of 12 ml each) of EcoRI digested liver DNA (17) were precipitated with ethanol, separated on a 0.5 % agarose gel, transferred to a nitrocellulose filter and hybridized with the T3 subclone (Fig. 2c) nick-translated to a specific activity of $1x10^8$ cpm/µg. After hybridization the two final washes were with 1xSSC, 0.5 % SDS at 68^o for 15 min each. Autoradiography was with an intensifying screen overnight. For details of the hybridization marker and the hybridization procedure see legend to Fig. 1 of (8). 5 µg of EcoRI digested total liver DNA were analyzed in parallel. Fraction numbers are indicated. Sizes are in kb.

the rearranged C gene segment were identified in MOPC41 (3,20) and in MOPC321 (21). The EcoRI fragment found in MOPC21a DNA migrated as the 15 kb fragment from mouse liver. In the DNA of the lambda chain producing tumor RPC20, however, no hybridization of the C region probe was found indicating a deletion of this region on both homologous chromosomes.

The J region probe hybridized, as expected, to the same DNA fragments as the C region probe. However, as in myeloma T additional DNA fragments of smaller size were found in the DNA of MOPC41 and MOPC21a. No further band could be detected with the J region probe in MOPC321 DNA (this study) or in MPC11 DNA (22). In EcoRI digested DNA of the lambda chain producing myeloma RPC20 two fragments were found which hybridized to the J region probe, indicating that the deletion of the C region on both homologous chromosomes did not include all of the J region

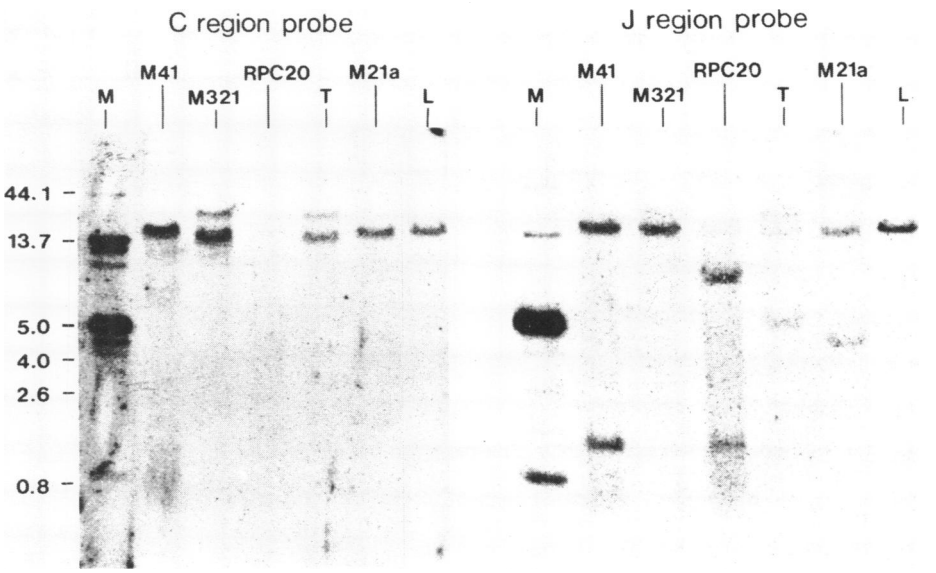

Figure 5. Filter hybridizations of EcoRI digested DNA from mouse myelomas (MOPC41, MOPC321, RPC20, T, MOPC21a) and from mouse liver (L) with the C region and J region probes. 10 µg of restricted DNA per slot was analyzed as described in Fig. 1 of (8) except that 100 ng/ml of the nick-translated plasmid probes (specific activity $4x10^7$ cpm/µg) were used for hybridization. The C region probe was a subclone in pBR322 of the 2.8 kb HindIII-BamHI fragment comprising the C gene segment of T1 (compare Fig. 2b of ref. 3). The J region subclone is the one shown in Fig. 2a of (8). Autoradiography at -80^{o} C with intensifying screens was for 36 h and 5 days for the C and J region experiments, respectively. Fragment sizes are given in kb.

sequences present in our subclone.

DISCUSSION

We have isolated from the DNA of myeloma T an EcoRI fragment which has been generated by a joining of flanking sequences of a kappa V gene segment and the J1 gene segment. This fragment, however, does not result directly from a combination of the flanking sequences of the V and J gene segments which are known to have been recombined in this myeloma DNA (8,18). Two findings support this contention. 1) The fragment T3 contains the 5' flanking region of J1 but not the flanking regions of J2 or J5 which would have been expected to be present if T3 were directly

derived from the V-J recombination events in this tumor. 2) The V region flanking sequences present on T3 apparently do not belong to the V gene segments which have been recombined to form the two kappa chain genes found in the DNA of myeloma T (8,18). It also should be mentioned that only T3 and not a second similarly rearranged fragment was detected in the DNA of myeloma T, although two V-J recombination events must have occurred.

It may be that the V-J rearrangement leading to the active allele takes place by a direct deletion mechanism (5) whereas a rearrangement as represented by T3 is somehow characteristic for an aberrant joining of immunoglobulin genes. It also cannot be excluded that sequences as in T3 are formed only in myelomas but not in normal lymphocytes.

The question remains how the fragment T3 has been generated and whether its occurrence tells us something about the V-J recombination mechanism. In the V-J joining process the flanking DNA sequences may be excised and linked. We have to consider the possibilities that such a DNA segment then remains in an extra-chromosomal state or that it is integrated into the chromosome. Complicated concerted mechanisms could be envisaged, as well as stepwise mechanisms. T3 may be formed in a step preceding the joining of the proper V and J gene segments or in a subsequent step. The finding of T3 indicates that the deletion model in its simplest form may not describe the V-J recombination mechanism fully.

Since fragments with the hybridization properties of the fragment T3 have been detected also in some other myeloma DNAs the formation of joined flanking sequences in the genome may be part of a general V-J joining mechanism. It is interesting to note that a fragment with the hybridization properties of T3 has been found also in MOPC41, a myeloma in which the formation of the kappa light chain gene involves the J1 gene segment (3). A detailed analysis of this and the other myeloma fragments with the hybridization properties of T3 may help in the clarification of the details of the V-J joining mechanism.

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