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DNA clones containing mouse immunoglobulin κ chain genes isolated by in vitro packaging into phage λ coats
(somatic gene rearrangement/intron/Southern gel blotting/R loop mapping)

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ABSTRACT Endonuclease EcoRI-digested DNAs from BALB/c mouse embryos and MOPC 321 (κ chain secreter) myeloma were fractionated by agarose gel electrophoresis, and the DNA fragments containing part or all of the MOPC 321 κ chain structural gene sequences were visualized by the Southern gel blotting technique, using as the hybridization probes pCRI plasmids containing all or part of the enzymatically synthesized cDNA transcripts of the MOPC 321 κ chain mRNA. The clear differences observed in the hybridization patterns of the two DNAs are in agreement with our previously reported results obtained with endonuclease BamHI and confirm that the sequence arrangement of κ chain gene segments is different in the embryonic and myeloma cells. We have cloned most of the κ-sequence-positive EcoRI DNA fragments in Charon 4A phage by using the highly efficient in vitro phage λ DNA packaging method, and we have characterized the cloned mouse DNA sequences by agarose gel blotting and R-loop mapping in electron microscopy. These studies identified, among others, one EcoRI DNA fragment which contains both variable and constant immunoglobulin κ gene sequences and is present in only the myeloma DNA. The two sequences are separated by a 2.8-kb λ intron. We tentatively conclude that the κ gene sequences on this DNA fragment underwent somatic rearrangement.

In order to study the organization, structure, and expression of immunoglobulin κ genes we have cloned DNA segments carrying such genes by in vitro recombination with phage λ DNA. In our earlier reports we described several DNA clones isolated from mouse embryos or from a λ1 chain-producing myeloma. These DNA clones contained either the variable or the constant, or the variable plus constant DNA sequences coding for λ1 or λ2 light chains (1-3). Extensive characterization of these λ chain gene clones by restriction enzyme digestion, by electron microscopy, and by DNA sequencing established, among other things, that the variable- and the constant-coding DNA segments that are distant in embryonic cells are brought into proximity by somatic recombination during differentiation of lymphocytes (3, 4). These DNA clones were all isolated by transfection of CaCl2-treated Echerichia coli (5) with recombinant DNAs constructed in vitro from mouse DNA fragments and the EK-2 phage, λWES (6). We here report isolation and partial characterization of DNA clones containing gene sequences for κ-type light chains. These DNA clones were isolated by the more efficient in vitro DNA packaging procedures (7) that use another EK-2 phage, Charon 4A (8). Among the κ gene clones isolated, one from the myeloma MOPC 321, a κ chain secreter, was shown by R-loop mapping to contain both the variable and constant regions of immunoglobulin L chains (Vκ and Cκ gene sequences) that were separated by a 2.8-kb intron (untranslated DNA). Analysis of total cellular DNA by the Southern gel blotting procedure (9) showed that the myeloma DNA, but not the embryo DNA, contains a κ-sequence-positive DNA fragment of the same size as this cloned DNA. We conclude that the κ gene sequences in this DNA fragment are those that have gone through the somatic rearrangement process. The results thus extend our previous findings made with the λ gene sequences (2, 3).

MATERIALS AND METHODS

Bacteria and Phages. Phage Charon 4A and E. coli DP50 (Su II*, Su III*) were from F. Blattner of the University of Wisconsin, Madison (7). E. coli 803 (r5, m5, Su III*) was originally from K. and N. E. Murray of the University of Edinburgh. Lysozymes were used for preparation of packaging mixtures, BHB 2688 [N205 recA- (λ iimm434 cts t2 red3 Eam4 Sam7/λ)] and BHB 2690 [N205 recA- (λ iimm434 cts b2 red3 Dam15 Sam7/λ)] are from our stock.

In Vitro Packaging of Recombinant DNA. The procedures described by Collins and Hohn (7) were followed.

The two packaging mixtures used in the present experiments were assayed without the addition of exogenous λ DNA, and the ratio of plaque-forming units without addition (endogenous virus) to plaque-forming units with exogenous DNA (0.1 μg of λb2 DNA in the standard packaging mixture) was in both cases less than 10^-7, whereas the packaging efficiency of λb2 DNA was 1-2 x 10^8 plaque-forming units per μg of DNA. In addition, when an EK2 vector was packaged, the ratio of am+ phage (recombinants) to total phage was less than 7 x 10^-7. These characteristics of the packaging mixtures satisfy the EK-2 requirements set by the National Institutes of Health Recombinant DNA Molecule Program Advisory Committee.

Screening of Phage Plaques. An aliquot of the packaged phage was plated on the E. coli 803 host in a 20 x 20 cm- plastic dish (Nunc) so that 20,000-50,000 plaques would arise. Plaques were transferred onto a 19 x 19-cm membrane filter (Schleicher and Schuell) and processed by the method of Benton and Davis (10). The hybridization probe used was nick-translated DNA of the plasmid 5D10 carrying an 890-base pair sequence complementary to a purified MOPC 321 κ mRNA (M. Hirama, G. Matthysse, and S. Tonegawa, unpublished data). The details of the hybridization, washing, and autoradiography procedures will be described elsewhere.

Gel Blotting of the Cellular and Cloned DNA Fragments. The procedure developed by Southern (9) was followed with some minor modifications (3). A nick-translated Hha I fragment of plasmid 5D10 or 4D3 was used as the hybridization probe. The latter plasmid contains a 556-base-long DNA sequence that is complementary to the 3'-end half [minus poly(A)] of MOPC 321 κ mRNA and serves as a hybridization probe exclusively for the Cκ sequence (M. Hirama, G. Matthysse, and S. Tonegawa, unpublished data).

Abbreviations: kb, kbase; Vκ and Cκ, variable and constant regions of immunoglobulin κ chains.

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Other Procedures. Purification of mRNA (11), preparative agarose gel electrophoresis (12), detection of 5' DNA sequences in gel fractions (12), analytical agarose gel electrophoresis (13), ligation of DNA fragments (2), and electron microscopy of R-loops (2) were all described previously. Left and right arms of Charon 4A DNA were isolated by preparative agarose gel electrophoresis and used in ligation reactions.

All cloning experiments were carried out under P3-EK2 conditions in accordance with the National Institutes of Health recombinant DNA guidelines issued in June 1976.

RESULTS

\( \kappa \) Gene-Positive EcoRI DNA Fragments in the Total DNAs from Embryos and MOPC 321 Myelomas. Results of the Southern gel blotting experiments are shown in Fig. 1. When the nick-translated 5D10 plasmid, a probe for the \( V_\kappa \) plus the \( C_\kappa \) gene sequences, was used in hybridization, multiple bands appeared in the autoradiograms of both embryo and myeloma DNAs. The band patterns of the two DNAs are, however, clearly different: the myeloma DNA generates two extra bands of 22 and 14 kb. The pattern difference between the two DNAs was more dramatically seen when the 4D5 plasmid, a probe for the \( C_\kappa \) gene sequence, was used in hybridization. This is because the \( C_\kappa \) sequence probe hybridized with fewer DNA fragments: only one from the embryo DNA (16 kb) and three from the myeloma DNA (22, 16, and 14 kb). This pattern difference is thus in accord with our earlier studies (12) and supports the conclusion that the arrangement of the \( \kappa \) gene sequences is different in the two DNAs.

Because the 10, 6, and 3.7 kb fragments identified by the \( V_\kappa \) plus \( C_\kappa \) probe did not hybridize with the \( C_\kappa \) probe, they should contain sequences homologous to the \( V_\kappa \) part. Other DNA fragments (22, 16, and 14 kb) hybridized with both of the DNA probes and, therefore, they should contain either both the \( V_\kappa \) and \( C_\kappa \) sequences or the \( C_\kappa \) sequence alone. In addition, there may be another DNA fragment that is about 22 kb long and contains sequences homologous only to the \( V_\kappa \) half of the 5D10 probe. (See the faint band at 22 kb in the left channel of Fig. 1 center. No band is visible at this position in the left channel of Fig. 1 right).

The band intensity was different among multiple DNA

components. Relatively weak hybridization in the 22-kb fragments can be ascribed to poor transfer efficiency of the large DNA fragments. The weaker hybridization in the smaller DNA fragments—10, 6, and 3.7 kb—particularly in the last two, compared to that in the larger DNA components such as the 16-kb fragment, would not be similarly explained, because transfer efficiency in the former size range should be equal to or better than that in the latter (9). A probable explanation is that the sequences contained in these smaller DNA fragments are not perfectly homologous to the MOPC 321 \( V_\kappa \) sequence.

Efficiency of the In Vitro DNA Packaging and the Scale of the Cloning Experiments. Various \( \kappa \) gene-positive DNA fragments were enriched 10- to 100-fold by preparative agarose gel electrophoresis and used as the source of mouse DNA in the cloning experiments (Table 1). Because the \( C_\kappa \) gene and each kind of the multiple \( V_\kappa \) genes are reiterated no more than a few times (11, 14), we expect to obtain one DNA clone of a particular \( \kappa \) gene sequence for approximately every 10\(^8\)–10\(^9\) independently arising clones. (Mouse genome size is about 2 \times 10\(^12\) daltons, whereas the average EcoRI DNA fragment is roughly 5 \times 10\(^6\) daltons). In comparison with the widely used transfection method, in vitro packaging of phage \( \lambda \) DNA (7, 15) gives 50- to 100-fold more plaques per unit weight or recombined DNA, as shown in Table 1, we could obtain 1–3 \times 10\(^6\) plaques per pmol of mouse DNA fragments used in ligation and packaging. This compares very favorably with the transfection efficiency of about 4 \times 10\(^4\) plaques per pmol obtained by us (1) or about 2 \times 10\(^4\) plaques per pmol calculated from the data published by others (16). It was thus possible to obtain 12 independent clones by screening 1.5 \times 10\(^5\) plaques generated by 0.15 pmol (~2 \mu g) of the 16–22 kb myeloma DNA fragments (Table 1, Exp. 1). Similarly, nine clones of apparently one kind were obtained by screening 1.9 \times 10\(^5\) plaques generated by 0.1 pmol (~0.6 \mu g) of the 10-kb embryo DNA fragments (Table 1, Exp. 2).

The screening was carried out by the rapid in situ hybridization method developed by Benton and Davis (10). By plating 2–5 \times 10\(^4\) plaques in a single large plastic plate (20 \times 20 cm) the 1–2 \times 10\(^5\) packaged phages to be processed for the isolation of DNA clones containing a particular type of \( \kappa \) gene could be handled with no more than a few plates and as few filters.

Partial Characterization of the Isolated \( \kappa \) Gene Clones. We analyzed the \( \kappa \) gene-positive DNA clones by agarose gel electrophoresis, by Southern gel blotting, and by R-loop mapping in order to determine the length of the inserted mouse DNA fragment, the type of Ig sequences (\( V_\kappa \), \( C_\kappa \) or \( V_\kappa \), with \( C_\kappa \) and the location of the \( \kappa \) sequences. The results of these experiments are summarized in Table 2. Each of the 12 clones isolated from the myeloma 16–22 kb DNA fraction fell into one of the four classes represented by Ig 25x, Ig 14x, Ig 45x, and Ig 24x. In addition, we isolated two clones from the 14-kb fraction (represented by Ig 31x) and one clone, Ig 21x, from the 10-kb fraction of myeloma DNA. The two embryonic DNA clones isolated from the 16-kb fraction—Ig 112x and Ig 146x—are indistinguishable from the myeloma classes Ig 45x and Ig 24x, respectively. Similarly, all clones such as Ig 4x, from embryonic 10-kb fractions seem to be identical with the respective myeloma class Ig 21x. By the criteria used in this preliminary characterization, we can, therefore, group the \( \kappa \) gene-positive clones into six classes which will be briefly described below.

(1) Ig 25x class. Agarose gel electrophoresis of EcoRI-digested DNA, as well as electron microscopy, showed that these five clones contained mouse DNA fragments of 21.5 kb. Because the inserted fragments hybridize to both whole and \( C_\kappa \) sequence probes, we conclude that this class of myeloma DNA fragments contains either both \( V_\kappa \) and \( C_\kappa \) sequences or only the \( C_\kappa \) sequence. The former assumption was proven to be correct by R-loop formation with MOPC 321 mRNA. The R-loop mole-
Table 1. Cloning of κ gene-positive fragments by in vitro packaging of recombinant DNA molecules

<table>
<thead>
<tr>
<th>Mouse DNA source</th>
<th>DNA in ligation mix</th>
<th>Plaques × 10^-3</th>
<th>Packaging efficiency, plaques × 10^-4 per mouse DNA quantity</th>
<th>Total plaques × 10^-5 screened</th>
<th>Positive plaques</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse, µg/ml</td>
<td>Vector, µg/ml</td>
<td>obtained by standard size packaging*</td>
<td>µg</td>
<td>pmol¹</td>
</tr>
</tbody>
</table>
| Exp. 1
Embryo, ~16 kb  | 20                 | 60              | 10.0                                                        | 10.0             | 110             |
| MOPC 321,        | 20                 | 60              | 13.3                                                        | 8.8              | 97              |
| 16 to 22 kb      | 30                 | 60              | 9.7                                                         | 9.7              | 130             |
| None             | 0                  | 60              | 7.0                                                         | 4.7              | 62              |
|                  |                    |                 |                                                             |                  |                 |
| Exp. 2
Embryo, ~10 kb  | 20                 | 60              | 28.0                                                        | 28.0             | 170             |
| MOPC 321,        | 20                 | 60              | 50.0                                                        | 50.0             | 300             |
| ~10 kb           |                    |                 |                                                             |                  |                 |
| None             | 0                  | 60              | 0.1                                                         |                  |                 |

Experiments 1 and 2 were carried out with two different preparations of packaging mixtures (Materials and Methods).
* Standard size packaging was carried out by using 40 µl of packaging mixture and 5 µl of ligated DNA.
† The average size of the 16- to 22-kb DNA fraction was considered to be 19 kb.
‡ Detection of the positive phages in this part of the experiment was not carried out under optimal conditions.

Molecules display a triple-loop structure similar to the one we described for the Ig 303λ clone isolated from a λ chain synthesizing myeloma (2). In the Ig 25κ type clones, two R-loops of 0.4 and 0.45 kb length are separated by a 2.8-kb double-stranded DNA intron loop. The R-loops lie 9.5 kb from one end of the DNA fragment (Fig. 2).

(ii) Ig 14κ class. Only one clone of this type was isolated from myeloma DNA. It carries an 18.5-kb fragment that hybridized with both κ gene probes. When hybridized with MOPC 321 mRNA, these molecules formed a single R-loop of 0.45 kb, which has one long RNA tail of ~0.4 kb and sometimes a shorter RNA tail at the other end (Fig. 2). Because this fragment hybridized also to the Cκ probe, we conclude that the observed R-loop corresponds to the Cκ gene sequence, the long RNA tail to the nonhybridized Vκ part, and the short tail to the poly(A) tail at the 3' end of the mRNA. The position and the size of this

Table 2. List of κ chain gene clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>N*</th>
<th>DNA source</th>
<th>Size</th>
<th>R-loops</th>
<th>H, (V + C)/C*</th>
<th>Genes¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ig 25κ</td>
<td>5</td>
<td>MOPC 321</td>
<td>21.5 kb</td>
<td>2 loops, 0.4/0.45 kb + intron, 2.8 kb</td>
<td>+/-</td>
<td>Vκ + Cκ</td>
</tr>
<tr>
<td>Ig 14κ</td>
<td>1</td>
<td>MOPC 321</td>
<td>18.5 kb</td>
<td>1 loop, 0.45 kb + RNA tail</td>
<td>+/-</td>
<td>Cκ</td>
</tr>
<tr>
<td>Ig 45κ</td>
<td>3</td>
<td>MOPC 321</td>
<td>16 kb</td>
<td>1 loop, 0.4 kb + RNA tail</td>
<td>+/-</td>
<td>Vκ</td>
</tr>
<tr>
<td>Ig 24κ</td>
<td>3</td>
<td>MOPC 321</td>
<td>15 kb</td>
<td>1 loop, 0.45 kb + RNA tails</td>
<td>+/-</td>
<td>Cκ</td>
</tr>
<tr>
<td>Ig 31κ</td>
<td>2</td>
<td>MOPC 321</td>
<td>14 kb</td>
<td>1 loop, 0.45 kb + RNA tail</td>
<td>+/-</td>
<td>Cκ</td>
</tr>
<tr>
<td>Ig 21κ</td>
<td>1</td>
<td>MOPC 321</td>
<td>9.5 kb</td>
<td>1 loop, 0.35 kb + RNA tail</td>
<td>+/-</td>
<td>Vκ</td>
</tr>
<tr>
<td>Ig 112κ</td>
<td>2</td>
<td>Embryo</td>
<td>16 kb</td>
<td>1 loop, 0.35 kb + RNA tail</td>
<td>+/-</td>
<td>Vκ</td>
</tr>
<tr>
<td>Ig 146κ</td>
<td>2</td>
<td>Embryo</td>
<td>15 kb</td>
<td>1 loop, 0.45 kb + RNA tail</td>
<td>+/-</td>
<td>Cκ</td>
</tr>
<tr>
<td>Ig 4κ</td>
<td>5</td>
<td>Embryo</td>
<td>9.5 kb</td>
<td>1 loop, 0.35 kb + RNA tail</td>
<td>+/-</td>
<td>Vκ</td>
</tr>
</tbody>
</table>

* N = number of independent isolates.
† H, (V + C)/C = Southern hybridization with V + C probe or with C probe.
‡ Types of κ Ig sequences contained.
(iii) Ig 45c class. Three clones isolated from myeloma DNA contained a 16-kb fragment that hybridizes only to the Vc+ Cγ probe and forms a single R-loop of 0.4 kb with a long RNA tail. The R-loop lies approximately in the middle of the DNA fragment, 8.2 kb from one end of the molecule. This loop probably corresponds to the Vc sequence and the RNA tail to the Cγ part of the mRNA molecule.

(iv) Ig 24c class. The three members of this class contained a 15-kb DNA fragment that was positive with both hybridization probes. R-loop mapping showed again a single R-loop of 0.45 kb, carrying an RNA tail, which lies 9.5 kb from one end of the molecule. These fragments therefore seem to carry only Cγ gene sequences.

(v) Ig 31c class. The size of the two clones isolated from the 14-kb myeloma DNA fitted well with that of the uncloned myeloma DNA fragment. A single R-loop containing Cγ gene sequences was located at 9.5 kb from one end (Fig. 2).

(vi) Ig 21c class. One clone was isolated from myeloma DNA of the 10-kb agarose gel fraction. This DNA fragment is 9.5 kb long and hybridized with the whole κ probe, but not with the Cγ probe. It therefore contains the Vc or Vc-like sequence. Hybridization with MOPC 321 κ mRNA yielded single R-loops of 0.35 kb that lie 8.2 kb from one end.

All embryos DNA clones represented by Ig 112c (16 kb) and Ig 146c (15 kb) seem to be similar to the corresponding size classes of the myeloma DNA Ig 45c and Ig 24c, respectively, both in hybridization properties and in size and position of R-loops (Fig. 2). The five embryonic 9.5-kb fragments of the Ig 4κ class are comparable to the corresponding myeloma fragment Ig 21c (Fig. 2).

All clones displayed only one particular kind of R-loop pattern; we never observed multiple loops on a single DNA fragment that would indicate the presence of multiple copies of a particular type of the κ sequences.

**DISCUSSION**

The efficiency of the in vitro packaging method is at least one, and usually two, orders of magnitude greater than that of transfection. We now obtain over 10⁶ plaqueforms by packaging 0.1 μg of mouse DNA fragments. This scale of packaging is sufficient for the cloning of a unique mammalian gene, if the DNA preparation used is 10- to 20-fold enriched for the particular gene sequence. The combined use of preparative agarose gel electrophoresis, in vitro packaging, and the Bent and Davis plaque screening technique (17) allows cloning of essentially any unique mammalian gene, for which hybridization probes are available, by handling a few micrograms of total cellular DNA, less than 100 μl of the packaging mix, and one or two large agar plate(s). Because the entire experiment is done on quite a small scale, it is easy to contain.

The results of the Southern gel blots of EcoRI-digested embryo and MOPC 321 myeloma DNA are in agreement with the notion (13, 18) that mouse κ gene sequences are somatically rearranged. Gene rearrangement during development of a chain-producing plasma cells was directly confirmed, and some details of the somatic rearrangement uncovered, by characterization of cloned DNA fragments carrying parts of the whole of a full λc type gene (3). The goal of the present study was to extend gene cloning to κ chains and thereby investigate, in an equally direct manner, the putative somatic rearrangement in this gene system. In the mouse, sequence diversity is much greater in Vκ than in Vλ regions (17). We expect this diversity difference of the two V region types to be reflected in the structural genes. Our previous hybridization kinetic studies of both κ and λ genes suggested that a group of highly homologous V regions, which are best approximated to the subgroup as defined by Cohn and his coworkers (18), are encoded by no
more than a few germ line genes (1, 19). Subsequent gel blotting experiments with the λ gene probe were consistent with this (unpublished data); all V, regions associated with type λA chains that belong to a single subgroup seem to share a single germ line V gene. Although there is no direct estimate of the total number of germ line V, genes, the one subgroup = one germ line V gene concept would predict as many as a hundred or so germ line V, genes (18, 20). If this hypothesis is correct, a single κ chain mRNA or cDNA would hybridize not only with its own germ line V, gene but also with those few V, genes that code for V regions of different, but sufficiently homologous, subgroups. The V genes for MOPC 321 and MOPC 70E V, regions may be an example (19) of this. In agreement with the above considerations, the Southern blot pattern of the MOPC 321 κ probe is more complex than that of the H 2020 λ probe. The major source of complexity is the appearance in the κ pattern of multiple bands in which hybridization took place in the V half of the probe, suggesting that the whole κ probe crosshybridized with several incompletely homologous V, genes. The lower degree of hybridization in some of these bands is consistent with this interpretation.

The results indicate that a mouse genome carries five or six MOPC 321-like germ line V genes. The number is only slightly higher than that obtained by hybridization kinetics (11) and is within the range of accuracy expected by the method. We have isolated and cloned DNA fragments representing most of the κ gene-positive EcoRI fragments identified by the agarose gel blotting technique. From embryo DNA three classes of clones were isolated, one class (Ig 146k) from the 16-kb DNA fraction which contains only C, sequences, and two classes, the Ig 112 k type from the 16-kb fraction and the Ig 4k type from the 10-kb fraction, which contain only V, sequences. We have not yet attempted to clone the smaller fragments that seem to have partial homology to the MOPC 321 V, sequence. The same three classes of DNA fragments were isolated also from MOPC 321 DNA. In addition, we cloned three DNA fragments which were absent in embryo DNA: the 14-kb Ig 31k, the 18.5-kb Ig 14k, and the 21.5-kb Ig 25k fragments. Only one representative of the 18.5-kb fragment was isolated and it contains only C, sequences as revealed with the MOPC 321 probe. This size of DNA fragment cannot be correlated to any visible hybridization-positive band in the EcoRI digested myeloma DNA, nor is any similar fragment visible in embryonic DNA (Fig. 1). Because of this and because of its relatively low frequency (only one clone of the Ig 14k type, compared to three to five clones of each other type), we think that this fragment is only a minor component of the DNA and may not be involved in the synthesis of MOPC 321 light chain polypeptides. The 14-kb DNA fragment clones in the Ig 31k type has its counterpart in uncloned myeloma DNA (Fig. 1 right). Because this DNA fragment is absent in embryo DNA, it is a candidate for the rearranged active MOPC 321 type λ. However, this DNA fragment contains no V, sequence. On the other hand, the 21.5-kb Ig 25k type clones contain both V, and C, gene sequences as revealed by R-loop mapping, and the corresponding DNA fragment is present only in myeloma DNA. As in the case of the Ig 303A clone that we have isolated from H 2020 DNA, a λA type light chain-secreting myeloma (2), the V and C parts of the Ig gene are separated by an intron (a stretch of nontranslated DNA). In the λA clone this intron was 1.25 kb; in the present κ clones we found an intron of 2.8 kb separating V, and C, sequences. By analogy with the λ light chain system, we conclude that these are the κ sequences that have gone through a similar rearrangement process.

The origin and physiological significance of neither the 14-κ/fragment (represented by the clone Ig 31k) nor the 18.5-kb fragment (represented by the clone Ig 14k) are clear. In the case of λA chain genes, we observed only one additional DNA fragment in the myeloma DNA, and this fragment was shown to arise by somatic recombination (5). The presence of multiple κ sequence-positive fragments that have apparently gone through some sort of rearrangement, however, is not peculiar to MOPC 321 myeloma. Our recent studies indicate that several other κ producing myelomas such as MOPC 21 and LPC-1 also contain multiple κ sequence positive DNA fragments that are absent in embryo DNA (unpublished observations). Does the presence of the multiple DNA fragments reflect general aberrancy of DNA organization that is attributable to the tumorous nature of myeloma cells? We do not think that this is the case because DNA extracted from all λ chain-producing myelomas studied exhibited no κ sequence-positive DNA fragment that is absent in embryo DNA (unpublished observation). It thus seems that the enzymes that are responsible for the normal rearrangement of κ gene sequences are also, directly or indirectly, involved in the generation of apparently aberrant DNA fragments. Whether the latter event also occurs in normal lymphocytes or plasma cells remains to be seen.

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