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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 *Eco*RI-digested DNAs from BALB/c mouse embryos and MOPC 321 (a κ chain secretor) 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 *Bam*HI and confirms that the sequence arrangement of κ chain genes is different in the embryo and myeloma cells. We have cloned most of the κ-sequence-positive *Eco*RI 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 *Eco*RI DNA fragment which contains both variable and constant immunoglobulin κ-gene sequences and is present only in the myeloma DNA. The two sequences are separated by a 2.8-kbase 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 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 λ_I chain-producing myeloma. These DNA clones contained either the variable or the constant, or the variable plus constant DNA sequences coding for λ_I or λ_{II} 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 embryo cells are brought into proximity by somatic recombination during differentiation of lymphocytes (3, 4). These DNA clones were all isolated by transfection of $CaCl_2$-treated *Escherichia coli* (5) with recombinant DNAs constructed *in vitro* from mouse DNA fragments and the EK-2 phage, λ_{WES} (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 secretor, was shown by R-loop mapping to contain both the variable and constant regions of immunoglobulin L chains (V_κ and C_κ gene sequences) that were separated by a 2.8-kbase (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 (r_k^-, m_k^-, Su III$^+$) was originally from K. and N. E. Murray of the University of Edinburgh. Lysogenes used for preparation of packaging mixtures, BHB 2688 [N205 *recA*$^-$ (λ *imm434 cIts b2 red3 Eam4 Sam7*)/λ] and BHB 2690 [N205 *recA*$^-$ (λ *imm434 cIts 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 exogeneous 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 $1–2 \times 10^8$ 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 7×10^{-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 × 20 cm-plastic dish (Nunc) so that 20,000–50,000 plaques would arise. Plaques were transferred onto a 19 × 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. Matthyssens, 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 350-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 C_κ sequence (M. Hirama, G. Matthyssens, and S. Tonegawa, unpublished data).

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Abbreviations: kb, kbase; V_κ and C_κ, 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 κ 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

κ Gene-Positive *Eco*RI 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 $V_{\kappa 321}$ plus the C_κ 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 4D3 plasmid, a probe for the C_κ gene sequence, was used in hybridization. This is because the C_κ 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 κ gene sequences is different in the two DNAs.

Because the 10, 6, and 3.7 kb fragments identified by the V_κ plus C_κ probe did not hybridize with the C_κ probe, they should contain sequences homologous to the V_κ 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_κ and C_κ sequences or the C_κ sequence alone. In addition, there may be another DNA fragment that is about 22 kb long and contains sequences homologous only to the V_κ 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_κ sequence.

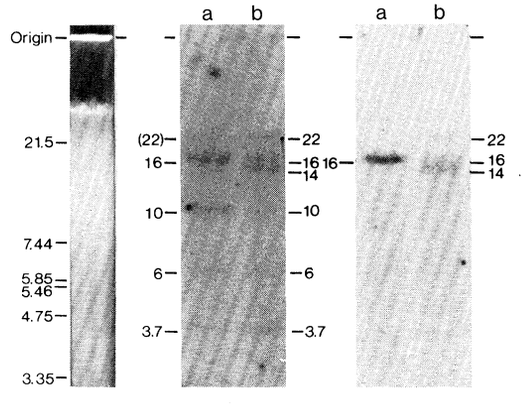

FIG. 1. κ-Chain gene-positive *Eco*RI-DNA fragments in total DNA of BALB/c mouse embryo and MOPC 321. (*Left*) Distribution of *Eco*RI DNA fragments of mouse embryos (12 days old) is shown by staining with ethidium bromide. *Eco*RI digestion of MOPC 321 myeloma DNA generated an essentially identical staining pattern. DNA fragments containing κ chain gene sequences were detected by incubation of the gel blots with two hybridization probes, (*Center*) plasmid 5D10, A $V_{\kappa M321}$ + C_κ probe and (*Right*) plasmid 4D3, a C_κ probe. The numbers designate size of DNA fragments (in kb pairs) as determined by *Eco*RI-λ phage DNA fragments electrophoresed in parallel. a, Embryo DNA; b, MOPC 321 myeloma DNA.

Efficiency of the *In Vitro* DNA Packaging and the Scale of the Cloning Experiments. Various κ 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_κ gene and each kind of the multiple V_κ genes are reiterated no more than a few times (11, 14), we expect to obtain one DNA clone of a particular κ gene sequence for approximately every 10^4–10^5 independently arising clones. (Mouse genome size is about 2×10^{12} daltons, whereas the average *Eco*RI DNA fragment is roughly 3×10^6 daltons). In comparison with the widely used transfection method, *in vitro* packaging of phage λ 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 4×10^4 plaques per pmol obtained by us (1) or about 2×10^4 plaques per pmol calculated from the data published by others (16). It was thus possible to obtain 12 independent clones by screening 1.5×10^5 plaques generated by 0.15 pmol (~2 μg) of the 16–22 kb myeloma DNA fragments (Table 1, Exp. 1). Similarly, nine clones of apparently one kind were obtained by screening 1.9×10^5 plaques generated by 0.1 pmol (~0.6 μg) 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 × 20 cm) the $1–2 \times 10^5$ packaged phages to be processed for the isolation of DNA clones containing a particular type of κ gene could be handled with no more than a few plates and as few filters.

Partial Characterization of the Isolated κ Gene Clones. We analyzed the κ 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_κ, C_κ, or $V_\kappa + C_\kappa$), and the location of the κ 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 25κ, Ig 14κ, Ig 45κ, and Ig 24κ. In addition, we isolated two clones from the 14-kb fraction (represented by Ig 31κ) and one clone, Ig 21κ, from the 10-kb fraction of myeloma DNA. The two embryonic DNA clones isolated from the 16-kb fraction—Ig 112κ and Ig 146κ—are indistinguishable from the myeloma classes Ig 45κ and Ig 24κ, respectively. Similarly, all clones such as Ig 4κ, from embryonic 10-kb fractions seem to be identical with the respective myeloma class Ig 21κ. By the criteria used in this preliminary characterization, we can, therefore, group the κ gene-positive clones into six classes which will be briefly described below.

(*i*) *Ig 25κ class.* Agarose gel electrophoresis of *Eco*RI-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_κ sequence probes, we conclude that this class of myeloma DNA fragments contains either both V_κ and C_κ sequences or only the C_κ 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

Mouse DNA source	DNA in ligation mix: Mouse, μg/ml	DNA in ligation mix: Vector, μg/ml	Plaques × 10^{-3} obtained by standard size packaging*	Packaging efficiency, plaques × 10^{-4} per mouse DNA quantity: μg	Packaging efficiency, plaques × 10^{-4} per mouse DNA quantity: pmol†	Total plaques × 10^{-5} screened	Positive plaques
Exp.1							
Embryo, ~16 kb	20	60	10.0	10.0	110		
	30	60	13.3	8.8	97	1.46	2‡
MOPC 321,	20	60	9.7	9.7	130		
16 to 22 kb	30	60	7.0	4.7	62	1.50	12
None	0	60	0.5				
Exp. 2							
Embryo, ~10 kb							
	20	60	28.0	28.0	170	1.90	9
MOPC 321,							
~10 kb	20	60	50.0	50.0	300	2.10	3
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.

cules 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 C_κ probe, we conclude that the observed R-loop corresponds to the C_κ gene sequence, the long RNA tail to the nonhybridized V_κ 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

Clone	*N**	DNA source	Size	R-loops	H, (V + C)/C†	Genes‡
Ig 25κ	5	MOPC 321 16–22 kb	21.5 kb	2 loops, 0.4/0.45 kb + intron, 2.8 kb	+/+	$V_\kappa + C_\kappa$
Ig 14κ	1	MOPC 321 16–22 kb	18.5 kb	1 loop, 0.45 kb + RNA tail	+/+	C_κ
Ig 45κ	3	MOPC 321 16–22 kb	16 kb	1 loop, 0.4 kb + RNA tail	+/−	V_κ
Ig 24κ	3	MOPC 321 16–22 kb	15 kb	1 loop, 0.45 kb + RNA tails	+/+	C_κ
Ig 31κ	2	MOPC 321 ≈14 kb	14 kb	1 loop, 0.45 kb + RNA tail	+/+	C_κ
Ig 21κ	1	MOPC 321 ~10 kb	9.5 kb	1 loop, 0.35 kb + RNA tail	+/−	V_κ
Ig 112κ	2	Embryo ~16 kb	16 kb	1 loop, 0.35 kb + RNA tail	+/−	V_κ
Ig 146κ	2	Embryo ~16 kb	15 kb	1 loop, 0.45 kb + RNA tail	+/+	C_κ
Ig 4κ	5	Embryo ~10 kb	9.5 kb	1 loop, 0.35 kb + RNA tail	+/−	V_κ

* *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.

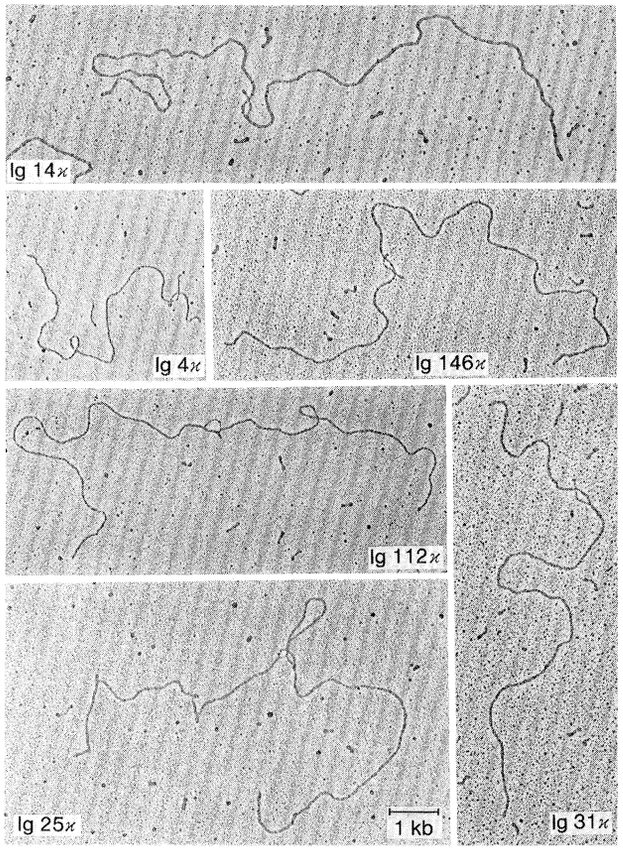

FIG. 2. Electron micrographs of R-loop molecules. *Eco*RI-digested DNA was incubated with MOPC 321 mRNA at molar ratios of mRNA/DNA varying from 20 to 70, at 57° for 8–14 hr in the following reaction mixture: 70% formamide/100 mM 1,4-piperazinediethanesulfonic acid (Pipes), pH 7.9/20 mM Tris·HCl/5 mM EDTA/0.56 M NaCl. Samples were spread for electron microscopy as described in ref. 11. The three myeloma DNA fragments representing the classes Ig 45κ, Ig 24κ, and Ig 21κ show identical R-loop patterns as the embryonic DNA fragments Ig 112κ, Ig 146κ, and Ig 4κ shown.

R-loop are similar to those of the longer R-loop observed on Ig 25κ type clones (9.5 kb from one end), thereby allowing us to orient these longer DNA fragments as well (see Fig. 3).

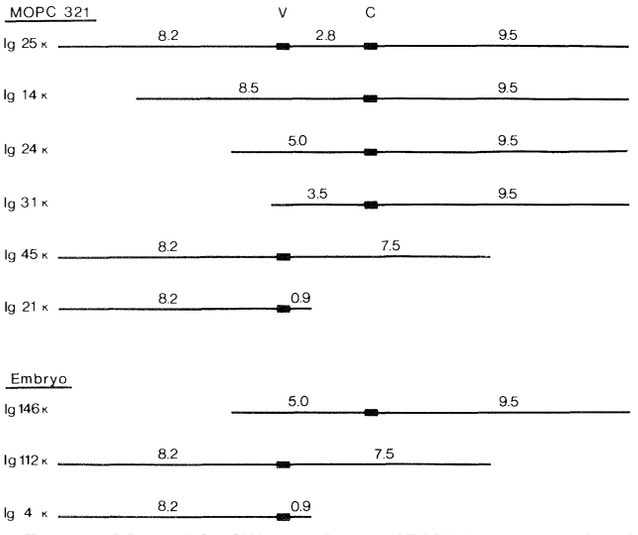

FIG. 3. Maps of the different classes of DNA fragments isolated from MOPC 321 and mouse embryo DNA. The black boxes show the location of κ gene sequences as determined by R-loop mapping. All lengths are given in kb pairs.

(*iii*) *Ig 45κ class.* Three clones isolated from myeloma DNA contained a 16-kb fragment that hybridizes only to the V_κ + C_κ 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 V_κ sequence and the RNA tail to the C_κ part of the mRNA molecule.

(*iv*) *Ig 24κ 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 C_κ gene sequences.

(*v*) *Ig 31κ 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 C_κ gene sequences was located at 9.5 kb from one end (Fig. 2).

(*vi*) *Ig 21κ 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 C_κ probe. It therefore contains the V_κ or V_κ-like sequence. Hybridization with MOPC 321κ mRNA yielded single R-loops of 0.35 kb that lie 8.2 kb from one end.

The embryonic DNA clones represented by Ig 112κ (16 kb) and Ig 146κ (15 kb) seem to be similar to the corresponding size classes of the myeloma DNAs Ig 45κ and Ig 24κ, 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 21κ (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 10^5 plaques 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 Benton 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 *Eco*RI-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 λ chain-producing plasma cells was directly confirmed, and some details of the somatic rearrangement uncovered, by characterization of cloned DNA fragments carrying parts or the whole of a full λ_I 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 V_κ than in V_λ 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_λ regions associated with type I λ 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_κ genes, the one subgroup = one germ line V gene concept would predict as many as a hundred or so germ line V_κ 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_κ gene but also with those few V_κ genes that code for V regions of different, but sufficiently homologous, subgroups. The V genes for MOPC 321 and MOPC 70E V_κ 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_κ 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 *Eco*RI fragments identified by the agarose gel blotting technique. From embryo DNA three classes of clones were isolated, one class (Ig 146κ) from the 16-kb DNA fraction which contains only C_κ sequences, and two classes, the Ig 112 κ type from the 16-kb fraction and the Ig 4κ type from the 10-kb fraction, which contain only V_κ sequences. We have not yet attempted to clone the smaller fragments that seem to have partial homology to the MOPC 321 V_κ 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 31κ, the 18.5-kb Ig 14κ, and the 21.5-kb Ig 25κ fragments. Only one representative of the 18.5-kb fragment was isolated and it contains only C_κ sequences as revealed with the MOPC 321 probe. This size of DNA fragment cannot be correlated to any visible hybridization-positive band in the *Eco*RI 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 14κ 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 31κ 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 κ gene. However, this DNA fragment contains no $V_{\kappa M321}$ sequence. On the other hand, the 21.5-kb Ig 25κ type clones contain both $V_{\kappa M321}$ and C_κ 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 303λ clone that we have isolated from H 2020 DNA, a λ_I 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 λ_I clone this intron was 1.25 kb; in the present κ clones we found an intron of 2.8 kb separating V_κ and C_κ 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-kb/fragment (represented by the clone lg 31κ) nor the 18.5-kb fragment (represented by the clone Ig 14κ) are clear. In the case of λ_I chain genes, we observed only one additional DNA fragment in the myeloma DNA, and this fragment was shown to arise by somatic recombination (3). 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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