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Recombinant DNA clones constructed from immunoglobulin kappa light chain messenger RNA

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ABSTRACT

Recombinant DNA clones have been generated from mouse myeloma MOPC 21 immunoglobulin κ light chain mRNA. Complementary DNA (cDNA) synthesized on κ light chain mRNA by reverse transcriptase was made double stranded and inserted into the bacterial plasmid vector,pMB9. Approximately 40 tetracycline-resistant transformed colonies containing κ light chain mRNA sequences were identified by colony hybridization. Five of these recombinant clones were selected and characterized. Three clones contain both κ light chain constant and variable region sequences. Two of these three recombinant clones have been shown to include all of the κ light chain constant and variable region coding sequences. Another of the five selected recombinant clones contain κ light chain constant region sequences. The remaining characterized clone appears to be derived from sequences at the 5'-end of κ light chain mRNA, possibly extending to the terminal cap structure.

INTRODUCTION

The origins of immunoglobulin diversity and the arrangement of immunoglobulin variable and constant region gene sequences in DNA constitute two of the most intriguing questions in eukaryotic molecular biology and immunology. The recently established techniques of recombinant DNA construction and molecular cloning afford a penetrating approach into these questions. We previously established general procedures which allow the construction of recombinant clones from any poly(A)-containing mRNA (1-3). Other laboratories, using similar experimental approaches, have independently established successful methods for the molecular cloning of poly(A)-containing mRNA (4-10). Such recombinant clones containing specific eukaryotic structural gene sequences (i.e., mRNA sequences) provide pure hybridization probes for the isolation and analysis of eukaryotic genes. We have constructed recombinant DNA clones from purified MOPC 21 immunoglobulin κ

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light chain mRNA and γ heavy chain mRNA. In this report we present our characterization of five selected recombinant clones containing κ light chain mRNA sequences. Using a combination of hybridization procedures and restriction enzyme mapping, we have determined that the combined sequences represented in the selected κ light chain clones described here include virtually the entire length of the κ light chain mRNA. The recombinant clones constructed from MOPC 21 γ heavy chain mRNA will be described elsewhere.

MATERIALS AND METHODS

Murine Myeloma Cells and mRNA Preparation. Immunoglobulin producing (IgG_1, κ), mouse myeloma MOPC 21 cells (11), were grown as solid tumors following subcutaneous injection of BALB/c mice. The P3 line of MOPC 21 cells adapted to tissue culture (12) was maintained and labelled in Dulbecco's modified Eagle's medium (13). Large scale preparations of polysomal RNA were prepared from frozen MOPC 21 solid tumors following cell disruption by nitrogen cavitation (13). Immunoglobulin κ light chain mRNA was purified from polysomal RNA by repeated oligo(dT) cellulose selections and sucrose gradient sedimentations (14-16). The κ light chain mRNA preparation used in constructing the clones described here was estimated to be approximately 50% pure by electrophoresis in 98% formamide gels (14-16). The synthesis of complementary DNA (cDNA) from purified κ light chain mRNA by reverse transcriptase was carried out using conditions which promote full length transcripts (1,3). Analysis of the light chain cDNA in 98% formamide gel electrophoresis (6) revealed that 50-60% migrated as full length transcripts of κ light chain mRNA (results not shown). Purified reverse transcriptase was obtained through the Office of Program Resources and Logistics, Viral Cancer Program, of the National Cancer Institute.

Synthesis of Double-stranded DNA. The reaction was carried out in a 120 μl volume for 15 minutes at 37^0C in 66 mM KH_2PO_4 (pH 7.4), 6.6 mM $MgCl_2$, 1 mM 2-mercaptoethanol with 1 μg immunoglobulin light chain cDNA, 7.6 units DNA polymerase and 4 nmol of each deoxynucleoside triphosphate. ^3H-labelled TTP (20 μCi) was included so that the reaction could be followed via acid-precipitable radioactive material. The reaction was self-priming due to a fold-back region at the 3'-ends of the cDNA molecules (1-3,6,7,17). Ten microliters of 1 M EDTA was added to stop the reaction, and after phenol extraction the aqueous layer was chromatographed through a Bio-Gel P60 column (0.6 x 15 cm) with 0.01 M triethylamine bicarbonate (pH 8.5). The excluded

peak contained in 0.5 ml was ethanol precipitated.

Cleavage of Double-Stranded Hairpin DNA. Cleavage of the hairpin loop joining the two strands of DNA and the removal of remaining single-stranded DNA was accomplished with S1 nuclease using conditions similar to Shenk et al (18). The duplex DNA (0.88 μg, calculated from the incorporation of ^{3}H label during second strand synthesis) was incubated with 119 units of S1 nuclease for 1 hr at room temperature in 200 μl of 0.28 M NaCl, 0.0045 M $ZnSO_4$, 0.03 M sodium acetate (pH 4.6). The reaction was terminated with the addition of 1/10 volume of 1 M Tris base. E. coli carrier tRNA (20 μg) was added and the mixture was then extracted with phenol. The aqueous phase was ethanol precipitated.

Attachment of Homopolymers to Immunoglobulin and Plasmid DNAs. Bacteriophage lambda exonuclease was used under conditions described by Lobban and Kaiser (19) to remove approximately 30 nucleotides from the 5'-OH ends of the linear double-stranded immunoglobulin light chain DNA and Eco RI cut pMB9 plasmid DNA molecules. The reaction was terminated by phenol extraction and the DNA was ethanol precipitated. We no longer use the lambda exonuclease treatment since it is not necessary for the addition of homopolymers by terminal transferase using the conditions described below for pMB9.

Addition of poly(dA) to exonuclease-treated immunoglobulin DNA with terminal transferase was carried out in 150 μl of 0.05 M KH_2PO_4 (pH adjusted to 6.9 with KOH), 9 mM $MgCl_2$, 1 mM 2-mercaptoethanol with immunoglobulin DNA at 3.3 μg/ml. 0.17 μCi of ^{32}P was present as the alpha phosphate in 14 nmol of dATP. Three hundred units of terminal transferase were added to initiate the reaction at 37°C. Poly(dT) was similarly added to lambda-exonuclease-treated Eco RI digested pMB9 plasmid DNA (20) except in 0.2 M cacodylic acid, adjusted to pH 7.2 with KOH, 1 mM $CoCl_2$, 2.5 mM 2-mercaptoethanol made fresh prior to each use. The reactions were terminated by phenol extraction, and the DNA was ethanol precipitated. The DNA was resuspended in 0.2 ml of 0.1 M NaCl, 0.01 M Tris pH 7.5 and passed over Bio-Gel agarose A - 5 m columns (100 ml/mg of DNA) with 0.1 M NaCl, 0.01 M Tris (pH 7.5). The fractions were assayed for acid-precipitable radioactive material and the peak fractions were pooled and ethanol precipitated.

Annealing and Transformation. Equimolar quantities of poly(dA)-tailed immunoglobulin DNA and poly(dT)-tailed pMB9 plasmid DNA were resuspended at 7 nM each (4 μg/ml of pMB9) in 10 mM Tris-HCl (pH 8.1), 100 mM $CaCl_2$, 1 mM EDTA, incubated at 51°C for 0.5 hr, and then allowed to cool slowly to room temperature over 3 hr. E. coli HB101 was transformed as

previously described (1,3). The annealed DNA mixture was added to an equal volume of .01 M Tris-HCl (pH 7.5) 0.02 M $CaCl_2$, 0.02 M $MgCl_2$. Twice this total volume of E. coli cells treated with 0.05 M $CaCl_2$ was then added. The mixture sat on ice for 15 min, was taken to 37°C for 4 min, and was left at room temperature for 10 min. The mixture was then added to a 3X concentrate of Luria broth and was incubated 0.5 hr at 37°C before being spread onto Luria broth agar plates containing 15 μg/ml of tetracycline. The transformant colonies were assayed for immunoglobulin mRNA sequences by the colony hybridization technique of Grunstein and Hogness (21) using ^{32}P-labelled cRNA made as in Poon et al. (22).

Biohazard Considerations.

The recombinant DNA clones described here were constructed from immunoglobulin κ light chain mRNA in a single cloning experiment carried out in December, 1975. This cloning was conducted in full compliance with the Asilomar Guidelines then in effect, using biocontainment conditions subsequently designated P3, EK1 under the NIH Guidelines for Research Involving Recombinant DNA Molecules issued in June, 1976. On the basis of our characterization of the five selected immunoglobulin κ light chain mRNA clones presented here (designated pL21-1 - pL21-5), the NIH Recombinant DNA Molecule Program Advisory Committee approved these clones for P3, EK1 Biocontainment in January, 1977.

RESULTS

Complementary DNA (cDNA) synthesized on purified MOPC 21 κ light chain mRNA by reverse transcriptase was made double-stranded, inserted into the plasmid vector pMB9 (20) and transformed into E. coli by procedures similar to those reported for the recombinant cloning of rabbit globin mRNA (1,3). Recombinant clones containing κ mRNA sequences were initially identified among the tetracycline resistant transformed colonies by Grunstein-Hogness colony hybridization using ^{32}P-cRNA prepared from the κ light chain cDNA used in constructing the clones (21,22). Of approximately 40 tet^r colonies giving a positive signal in this test, 5 (designated pL21-1 to pL21-5) showing the most definitive hybridization signal were selected for further characterization. Purified plasmid DNA was prepared from 100 ml cultures of these 5 clones and used for the following characterizations.

Immunoglobulin κ light chain mRNA comprised the most abundant species in the purified mRNA preparation used in this cloning. Accordingly,

transformed clones containing immunoglobulin κ light chain mRNA sequences should hybridize a substantial fraction of the cDNA made from this mRNA preparation. The results in Table I confirm this expectation. All five selected κ light chains hybridized and protected between 40-60% of the κ light chain cDNA from S_1 nuclease digestion.

Further evidence indicating that these selected clones contain κ light chain mRNA sequences has been obtained by the isolation of 13S κ light chain mRNA on columns containing purified recombinant plasmid DNA. Recombinant plasmid DNA linked to Sepharose has proven to be an efficient selection system for isolating intact immunoglobulin light chain mRNA (34). As shown in Table II, the specificity of this selection is quite high. The binding of heterologous RNA; including 18S and 28S HeLa rRNA, poly(A), and HeLa poly(A)-containing mRNA, ranges from 0.01 - 0.04% of the input radioactivity. Between 10-14% of three different preparations of poly(A)-containing mRNA from immunoglobulin producing MOPC 21 cells was selected on the recombinant plasmid DNA-sepharose. In all these tests and selections, the recombinant plasmid DNA was in substantial excess. The recombinant plasmid DNA-sepharose was also presaturated with a vast excess of unlabelled poly(A) to prevent binding of poly(A) sequences in mRNA with the poly(T) homopolymer tails present in the recombinant plasmid DNA.

TABLE I. Hybridization of MOPC 21 light chain cDNA with recombinant plasmid DNA from isolated recombinant clones.

RECOMBINANT PLASMID DNA	%INPUT cDNA HYBRIDIZED
pL21-1	58
pL21-2	51
pL21-3	37
pL21-4	53
pL21-5	42
pMB9	6

Hybridizations were carried out for 24 hr at 65°C in 200 μl of solution; 0.3 M NaCl, 0.02 Tes {N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid } (pH 7.4), 0.001 m EDTA containing 4 μg of cloned purified κ recombinant plasmid DNA or pMB9 and 3000 cpm of ^{3}H-cDNA (approximately 10^6 cpm/μg) used in constructing the κ light chain cDNA recombinant clones. Nuclease S_1 digestions were carried out as described by Vogt (23). Values presented are corrected for 10% S1 nuclease resistance in cDNA after boiling and rapid quenching (i.e., "snapback").

TABLE II. Selection of RNA on Cloned MOPC 21 Immunoglobulin κ light chain recombinant plasmid DNA.

RNA Sample	Input cpm	cpm hybridized and eluted	% Input cpm hybridized and eluted
18S rRNA	1.5×10^6	100	0.01
28S rRNA	1.5×10^6	100	0.01
poly(A)	5.6×10^5	240	0.04
HeLa poly(A)-containing mRNA	1.8×10^6	220	0.01
MOPC 21 poly(A)-containing mRNA			
prep 1	4×10^4	4,000	10
prep 2	3×10^5	41,000	13.6
prep 3	1.2×10^5	13,000	10.6

All hybrid-selections were carried out on 0.5 ml of sepharose containing 500 μg of purified, covalently-linked recombinant plasmid DNA. Purified denatured recombinant plasmid DNA from immunoglobulin light chain cDNA clone pL21-4 was used for all these hybridizations. Hybridizations with cloned plasmid-cDNA sepharose were carried out in silanized tubes for 6 hours at 37°C in 0.5 M NaCl, 0.25 M Tris HCl, 0.5% SDS, 0.001 M EDTA and 50% deionized formamide with continous gentle agitation. Prior to hybrid selection, the plasmid-cDNA sepharose was presaturated with 200 μg of poly(A). Following hybrid selection, the plasmid DNA-sepharose was transferred to a water-jacketed column for washing and elution. The column was first washed with 50 volumes of the hybridization buffer. The hybrid-selected RNA was then eluted with 5 volumes of 98% formamide in 0.01 M Tris (pH 7.5), 0.001 M EDTA, 0.1% SDS, followed by 5 column volumes of 1.0 M NaCl (24).

The RNA isolated from MOPC 21 poly(A)-containing mRNA migrated in 98% formamide gel electrophoresis as a single band (Fig. 1) with the molecular weight published for immunoglobulin κ light chain mRNA (reviewed in 25). This isolated 13S mRNA was translated in the _in vitro_ wheat germ protein synthesizing system to yield the pre-κ light chain polypeptide product (26-28) reported in this cell free system (results not shown). The isolation and translation of MOPC 21 κ light chain mRNA described here was carried out with recombinant plasmid pL21-4 DNA. However, identical results have also been obtained with DNA-column selections using κ light chain plasmid DNA from the other selected recombinant clones.

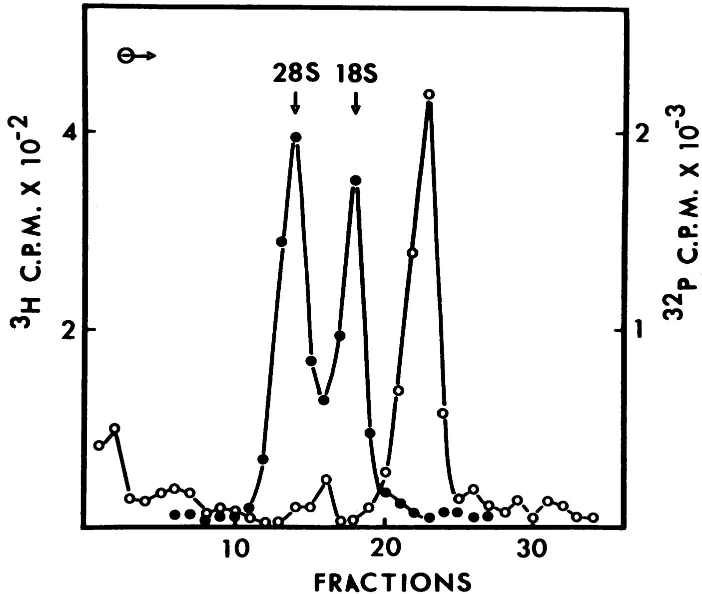


Figure 1. Analysis of κ light chain mRNA Isolated by Recombinant Plasmid DNA Column Selection. P3 ^{3}H-polysomal RNA was hybrid selected on PL21-4 DNA-Sepharose as described in Table II (—o—). Migration is shown in relation to 18S and 28S rRNA run in a parallel gel (—•—). Electrophoresis was carried out in 98% formamide-4% polyacrylamide tube gels at 100 volts, for 6 hr, in Tris-borate buffer (6,29).

An estimate of the length of the inserted sequences (including flanking dA:dT homopolymer "tails") was obtained by Hpa II restriction enzyme digestion and agarose gel electrophoresis in comparison to restriction digestion standards prepared from ϕX174 RF DNA (30). The single EcoRI cleavage site in pMB9 (20) at which the recombinant sequences were inserted lies within the largest Hpa II fragment (Hpa II_{1172}) generated from cleavage of the pMB9 plasmid vector (Fig. 2). The remaining pMB9 Hpa II restriction fragments are 510 base pairs and smaller.

In all the recombinant light chain clones, the Hpa II restriction fragments ≤510 base pairs correspond to pMB9 bands. The largest Hpa II digestion fragments from pL21-4 and pL21-5 are substantially larger than pMB9 Hpa II_{1172} and contain inserted regions of 530 and 830 base pairs respectively. The inserted κ light chain sequences in these two recombinant plasmids do not contain internal Hpa II sites. In contrast, pL21-1, pL21-2, and pL21-3 all contain a Hpa II cleavage site within the inserted sequences and exhibit two bands larger than 510 base pairs (Fig. 2). The estimated total length of the inserted sequences

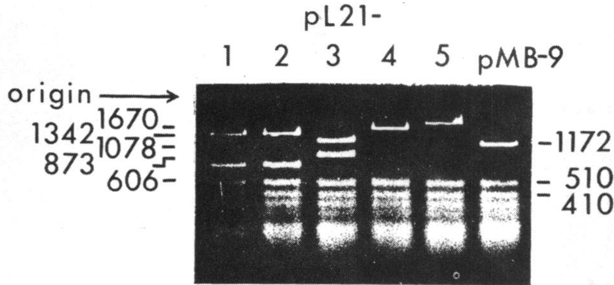


Figure 2. Size estimation of inserted κ light chain sequences in cloned recombinant plasmid DNA. Purified κ light chain recombinant plasmid DNA and pMB9 DNA were Hpa II digested and electrophoresed on a 3% horizontal agarose gel (20 x 20 x .2 cm, with agarose wicks) in tris-acetate-EDTA-NaCl buffer at 10V for 18hr. (31). Sizes were estimated from comparison with mobilities of known DNA fragments from restriction enzyme digestions of ΦX174 RF DNA (30) shown at the left.

(including homopolymer "tails") in these κ light chain recombinant plasmids is approximately 1070 for pL21-1 and pL21-2, and 910 for pL21-3 (See Table V).

The length of κ mRNA sequences and the size of the flanking homopolymer "tails" was estimated by electron microscope measurements of deletion loops resulting from heteroduplex formation between recombinant plasmid DNA and pMB9 plasmid vector DNA (see Fig. 3). The lengths of the inserted κ mRNA sequences and of the flanking homopolymer "tails" estimated by this approach are summarized in Table III. The size estimate for the κ mRNA sequences in pL21-1 are in good agreement with the value from detailed restriction mapping presented in the following paper (39). The stems are composed of flanking dA and T homopolymer stretches in duplex and represent minimum estimates reflecting the shorter of the two flanking homopolymer sequences. Heteroduplex mappings on globin mRNA clone PHb72 (32), where nucleotide sequencing has established the flanking homopolymer stretches to be 125 and 55 nucleotides, gave a stem value of 40 $\pm$ 36 nucleotide pairs.

Recombinant clones pL21-1 to pL21-3 contain sufficiently long inserted sequences to include both immunoglobulin variable (V) and (C) constant region coding sequences. The presence of constant region sequences in the κ light chain clones was estab-

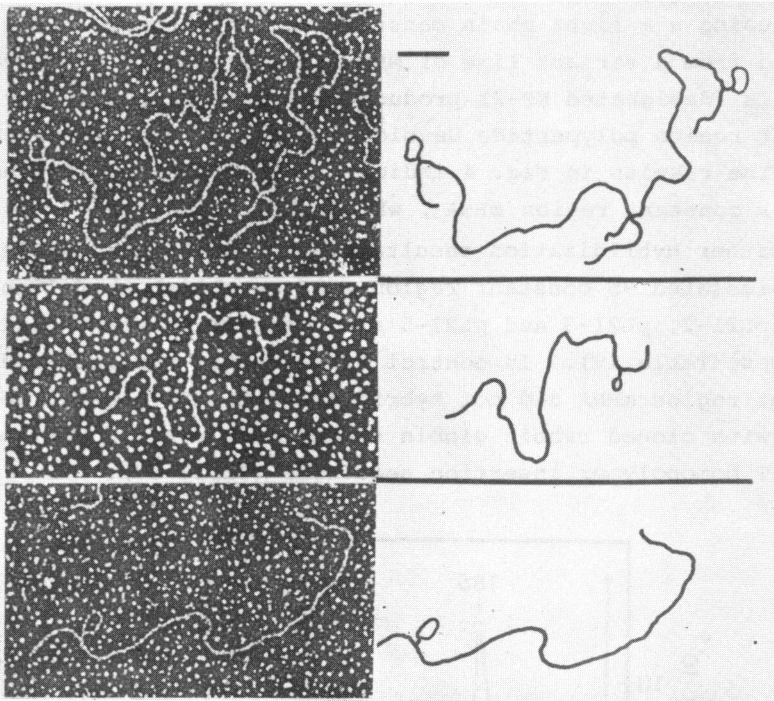
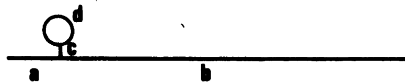


Figure 3. Heteroduplex Mapping of MOPC 21 Immunoglobulin κ light chain mRNA clones. Plasmid vector, pMB9, and κ light chain cloned DNAs were restricted with Bam HI, annealed to form heteroduplexes and then spread in 50% formamide for electron microscopy according to the procedures of Davis et. al. (33). Lengths were determined in relation to single and double-stranded ΦX174 DNA molecules on the same photographic negatives. The plates from the top are pL21-1, pL21-4 and pL21-5. The bar shown in the schematic representation on the right represents 0.2μ, equal to 560 nucleotide pairs or 630 nucleotides.

Table III. Summary of Heteroduplex Mapping of MOPC 21 Light Chain Recombinant DNA clones.



Heteroduplex	a	b	c*	d‡
	(Lengths in nucleotide pairs)			(nucleotides)
pL21-1/pMB9	726±97	4863±558	55±38	988±126
pl21-4/pMB9	608±75	4782±386	64±33	270±99
pL21-5/pMB9	655±8	4818±187	77±59	677±96

*c = estimated minimum size of flanking dA:T sequences
‡d = estimated κ mRNA sequences

lished using a κ light chain constant region specific mRNA obtained from a variant line of MPC-11 cells (34). These variant cells (designated NP-2) produce a 9-10S mRNA coding for a constant region polypeptide devoid of variable region sequences (35). The results in Fig. 4 indicate that pL21-1 hybridizes with the 9S κ constant region mRNA, while pL21-4 does not.

Further hybridization results using recombinant DNA-Sepharose column-isolated 9S constant region mRNA establish that light chain clones pL21-2, pL21-3 and pL21-5 also contain constant region sequences (Table IV). In control experiments, the isolated 9S constant region mRNA did not hybridize with pMB9 plasmid vector DNA or with cloned rabbit globin recombinant plasmid DNA containing dA:T homopolymer insertion sequences (Table IV).

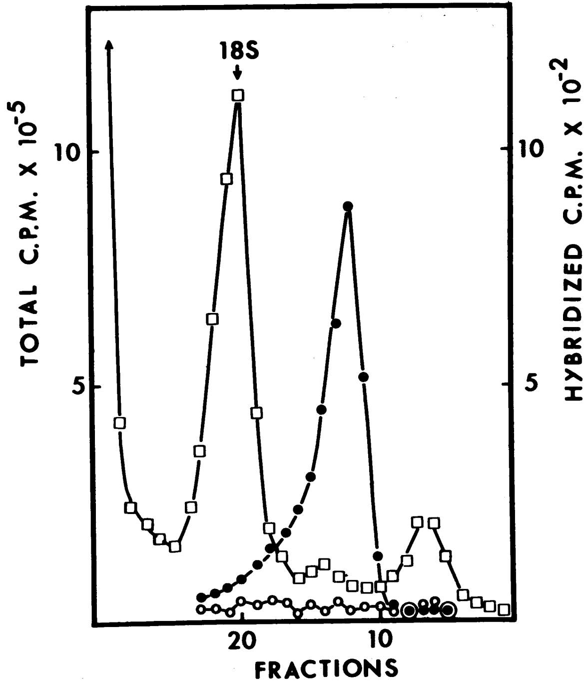


Figure 4. Hybridization of recombinant plasmid DNA with a 9S κ constant region mRNA. Cytoplasmic RNA isolated from NP-2 cells (13) was heated 3 minutes at 65°C and then layered on a 15-30% sucrose gradient and spun 16 hr. at 36,000 rpm in a Beckman SW 41 rotor (36). Isolated cytoplasmic RNA fractions (—□—) were exhaustively hybridized with either pL21-1 (—●—) or pL21-4 DNA filters (—○—) as described in Table IV.

Three of the light chain clones; pL21-1, pL21-2 and pL21-3, contain a Hpa II cleavage site located within the inserted κ mRNA sequences and yield two Hpa II restriction fragments containing inserted sequences (Fig. 2). The internal Hpa II cleavage site in the inserted sequences of these three light chain clones was localized to the MOPC 21 κ light chain variable region by the following considerations. First, all potential Hpa II cleavage sites in the MOPC 21 κ light chain constant region predicted from the amino acid sequence are eliminated by the published nucleotide sequence data (37). In addition, no Hpa II sites are present in the reported 3'-terminal sequences adjacent to poly (A) in κ light chain mRNA (37). Finally, if the Hpa II site is located in the MOPC 21 κ variable region, then only one of the two Hpa II digestion products of clones, pL21-1 to pL21-3, should hybridize with the κ constant region 9S mRNA from MPC-11. This prediction was confirmed by the hybridization of purified 9S κ constant region mRNA with only the larger of two isolated Hpa II fragments containing inserted sequences from pL21-1 (See Table IV). Detailed restriction enzyme mapping presented in the accompanying paper indicates that this Hpa II site is located at amino acid 53 in the MOPC 21 κ light chain variable region (38).

TABLE IV. Hybridization of DNA from MOPC 21 light chain recombinant clones with 9S mRNA coding for light chain constant region.

Recombinant Plasmid DNA	CPM Hybridized
pL21-1	279
pL21-2	240
pL21-3	175
pL21-4	28
pL21-5	208
pL21-1	
Hpa II_{1700}	233
Hpa II_{970}	40
pMB9	34
pHb72	28
blank	30

DNA filters containing 5μg of denatured plasmid DNA, or an amount equivalent to that in 5μg for the pL21-1 isolated Hpa II restriction fragments, were hybridized 20 hr. at 65°C with 400 ^{3}H-cpm of hybrid-isolated NP-2 9S κ constant region mRNA under conditions described in Table I. Hybridized cpm were scored following pancreatic and T_1 RNAse digestion (10).

A summary of the characteristics of these κ light chain mRNA recombinant clones is presented in Table V.

TABLE V. Summary of characteristics of recombinant DNA clones constructed from MOPC 21 immunoglobulin light chain messenger RNA.

Recombinant Plasmid	Total Inserted Sequence* (Nucleotide Pairs)	κ mRNA sequence (Nucleotides)	V-Region	C-Region
pL21-1	1070	990	+	+
pL21-2	1070		+	+
pL21-3	910		+	+
pL21-4	530	270	?	-
pL21-5	830	680	-	+

*Including Homopolymer "tails"

DISCUSSION

The combined data presented here indicate that the five selected recombinant clones, designated pL21-1 - pL21-5, contain MOPC 21 immunoglobulin κ light chain mRNA sequences. A detailed restriction map and nucleotide sequences for pL21-1 are presented in the accompanying paper (38). The cDNA used in constructing these κ light chain clones was synthesized under reverse transcriptase reaction conditions promoting long transcripts (1,3). Approximately 50-60% of the light chain cDNA appeared to be full length transcripts of κ light chain mRNA. In the construction of these clones, no attempt was made to select full length double-stranded molecules following complementary strand synthesis by DNA polymerase I and S_1 nuclease digestion. Provided that the DNA polymerase I reaction does not always produce full length complementary strands, this approach should produce clones from both the 3'- and 5'-portions of κ light chain mRNA as well as clones containing substantial or complete copies of κ mRNA. Our characterization of the κ light chain clones confirms this expectation. The three clones containing the largest inserted sequences, pL21-1 - pL21-3, are sufficiently large to include both the κ light chain variable and constant region sequences. The results presented in the accompanying paper confirm that immunoglobulin κ light chain

mRNA clone pL21-1 includes the entire 3'-untranslated region, the constant region and extends through the 5'-end of variable region into the leader coding sequences (38). From its size, recombinant plasmid clone, pL21-2 appears to be identical to pL21-1. Variable region specific clones have now been generated from recloning a Hpa II restriction fragment from pL21-1 (Komaromy, unpublished results). Plasmid clone pL21-5 clearly contains κ constant region sequences (Table IV). Given the lack of the Hpa II cleavage located in the middle of the κ light chain V-region in pL21-5, it appears that this clone contains less than 100-150 nucleotides of variable region sequence. Restriction mapping and nucleotide sequences support this prediction (P. Clarke and W. Salser, in preparation). Immunoglobulin recombinant clone pL21-4 does not contain constant region or 3'-untranslated region sequences (Fig. 4) nor does it contain the Hpa II and other restriction enzyme sites characteristic of the MOPC 21 variable region (Komaromy, unpublished results). Hybridization studies suggest that it shares a limited sequence overlap with the 5'-κ mRNA sequences in clone pL21-1. These preliminary findings suggest that pL21-4 represents the "leader" coding region and 5'-untranslated region sequences. From the estimated size of κ mRNA sequences in pL21-4 and the apparent minimal overlap with the "leader" coding sequences in pL21-1, it is possible that plasmid clone pL21-4 could extend to the 5'-end of the κ light chain mRNA, possibly including the 5'-terminal "cap" sequence. Nucleotide sequence analysis is now underway to further characterize the κ light chain mRNA sequences in this clone.

These characterized clones provide specific κ light chain structural gene sequence probes for the definitive analysis of immunoglobulin gene structure and expression. We have already used cloned immunoglobulin recombinant DNA to define the large nuclear RNA precursor and the processing steps leading to cytoplasmic κ light chain mRNA (36). We have now examined the arrangement of κ light chain variable and constant regions in myeloma cells by U.V. transcription mapping (reviewed in 39) and hybridization with these κ light chain clones (Gilmore-Hebert, Hercules, Komaromy and Wall, submitted for publication). These transcription mapping results reveal that κ light chain variable

and constant region coding sequences are widely separated in the DNA of myeloma cells.

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