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Rearrangement of Immunoglobulin Genes†

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ABSTRACT: The extent of rearrangements of immunoglobulin genes was investigated. The deoxyribonucleic acids (DNAs) of four κ-chain-producing myelomas (MOPC-167, MOPC-41, MOPC-21, and MOPC-321), one λ-chain myeloma (S178), Krebs ascites cells, liver, spleen, and thymus were digested with six different restriction endonucleases (*Kpn*I, *Eco*RI, *Pvu*II, *Xba*I, *Hin*dIII, and *Bam*HI) used alone or in all possible pairs. Following agarose gel electrophoresis and transfer to nitrocellulose filters [Southern, E. M. (1975) *J. Mol. Biol. 98*, 503], the DNAs were hybridized to one of three phosphorus-32 nick-translated probes: one for the κ-chain constant (C) region, one for the MOPC-167 κ-chain variable (V) region, and one for both the C and V regions. The probes were derived from a cloned complementary deoxyribonucleic acid from myeloma MOPC-167. It was found that liver, spleen, thymus and Krebs DNAs always produced hybridization band patterns indistinguishable from one another when a given enzyme or enzyme pair was used. One myeloma (MOPC-321) produced these same "germline" bands, but always exhibited other bands as well. The other four myelomas exhibited banding patterns which were not superimposable over the normal tissue pattern. Hybridization banding data obtained after double-enzyme digestion allowed the construction of restriction maps for sequences surrounding the C- and V-region genes in each of the DNAs. V maps for the MOPC-167 sequence were invariant in all DNAs except MOPC-167, indicating an absence of rearrangement in cells which do not express V_{167}. MOPC-167 showed single nongermline V and C maps which overlapped in such a way that the V- and C-containing fragments were about 1 kilobase (kb) apart. The 6 kb mapped at the 5′ side of the MOPC-167 V_{167} gene were identical with the sequence flanking the germline V_{167} gene. A single map, presumably in a germline configuration, was obtained for the sequences surrounding the C-region gene for the liver, spleen, thymus, and Krebs ascites cells. MOPC-321 had a map which was identical with this germline map, but it had in addition two rearranged C maps. The four other myelomas each gave one to three rearranged C maps, none of which were identical with any other C map. It was concluded that phenotypic "allelic exclusion" in myeloma cells is not necessarily correlated with the maintenance of one normal chromosome. A C-map comparison revealed that most restriction site alterations occur within 10 kb beyond the 5′ end of the restriction fragment carrying the C-region gene. Other features emerging from this study were the following. (1) All cells tested have multiple V_{167}-like sequences in identical restriction fragments. (2) S178, a λ myeloma, displays shifted κ genes. This correlates with our previous finding that S178 cells contain a low level of κ ribonucleic acid (RNA) [Storb, U., Hager, L., Wilson, R., & Putnam, D. (1977) *Biochemistry 16*, 5432]. There must therefore exist transcriptional or posttranscriptional controls in this tumor which limit the accumulation of κ mRNA but not λ mRNA. (3) The DNAs of spleen and thymus, organs which contain 87 and 99%, respectively, of cells which produce κ RNA [Storb, U. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2905], showed a germline restriction map for $C_κ$ genes, suggesting that the majority of κ genes are not rearranged in these cells or that one of the allelic $C_κ$ genes is maintained in a germline context. It is not yet known whether the differences between the myeloma cells tested and the nonmalignant lymphoid tissues are due to the polyploidy or to the differentiated plasma cell nature of the myeloma.

Studies of the genetics and protein structure of antibodies have led to the concept of "two genes–one polypeptide chain" (Dreyer & Bennett, 1965). The recent analyses of the organization of DNA sequences which contain immunoglobulin genes have supported this hypothesis and suggested that a rearrangement of sequences coding for different portions of an immunoglobulin chain has taken place in a cell which produces antibodies (Brack & Tonegawa, 1977; Brack et al., 1978; Lenhard-Schuller et al., 1978; Seidman et al., 1978a,b; Seidman & Leder, 1978; Bernard et al., 1978).

Cellular studies have shown that single lymphoid cells of an individual heterozygous for a given immunoglobulin allotype express only one allele, i.e. show "allelic exclusion" (Pernis

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et al., 1965). Previous studies on the DNA level have given ambiguous results as to whether allelic exclusion is determined before or after immunoglobulin gene rearrangement (Tonegawa et al., 1977). It appeared, therefore, important to survey several different cell types to determine whether and how C_κ[1] genes were rearranged. It was the aim of this study to determine the extent of rearrangements of genes coding for κ chains in cells which produce immunoglobulins and in cells which do not. DNAs from a large variety of cell types were analyzed in order to assess the arrangement of a particular V_κ gene, V_{167}, not known to be expressed by most of the cells tested except the myeloma MOPC-167, and to determine the arrangement of the C_κ genes. The alteration of restriction sites in the DNA sequences flanking V and C genes was taken as evidence for rearrangements which had occurred in the vicinity. It was expected that if allelic exclusion operated on the level of the DNA sequence that only expressed κ genes would be rearranged.

In the present study, the DNAs of five myelomas, Krebs ascites cells (derived from a mammary tumor), liver, spleen, and thymus were analyzed by restriction enzyme digestion and Southern (1975) blots. Six different enzymes were used either alone or in all possible pairs. The blots were analyzed by hybridization with a nick-translated cloned κ cDNA, either using the whole probe (V plus C) or using isolated V or C regions.

Experimental Procedures

Mouse Strains and Tumor Lines. In all experiments, organs were taken from and tumors were serially passaged in Balb/c mice, except for Krebs ascites cells which were passaged in Swiss mice. Five myeloma lines were used: MOPC-167, MOPC-41, MOPC-21, and MOPC-321, κ-chain producers obtained from Dr. M. Potter through Litton Bionetics, and S178, a λ-chain producer obtained from the Salk Institute.

DNA Preparation. DNA was prepared by using the method of Gross-Bellard et al. (1973) with some minor modifications. Tumors and organs were removed from the animal, rinsed in 0.15 M NaCl–10 mM phosphate, pH 6.8 (PBS), and then disrupted by using a motor-driven Teflon pestle. Krebs ascites cells were washed in PBS, concentrated by centrifugation, and suspended in 1 volume of PBS. The disrupted tumor or tissue or the Krebs ascites cell suspension was mixed with 20–40 volumes of a buffer: 10 mM NaCl, 10 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.5% sodium dodecyl sulfate, and 50 μg/mL proteinase K. After an overnight incubation at 37 °C, the mixture was extracted with phenol and subsequently dialyzed exhaustively against 15 mM NaCl and 2 mM EDTA. The DNA samples were treated with pancreatic RNase and then subjected to another digestion with proteinase K, an extraction with phenol, and finally exhaustive dialysis against 15 mM NaCl and 2 mM EDTA.

Restriction Endonuclease Digestion. The DNAs were digested with six restriction enzymes: *Kpn*I, *Eco*RI, *Pvu*II, *Xba*I, *Hin*dIII, and *Bam*HI, all obtained from New England Biolabs. The restriction enzyme digestion and extraction procedures with phenol and chloroform have been described (Wilson et al., 1978).

Blotting Technique and Hybridization. Restriction endonuclease cleaved DNAs were electrophoresed in 0.6% agarose gels in a buffer of 20 mM NaOAc, 40 mM Tris-acetate, pH 7.8, and 2 mM EDTA. Thirty micrograms of DNA was loaded into a well which had a cross section of 100 mm^2. The DNAs were denatured by soaking the gel in 0.4 M NaOH and 0.8 M NaCl for 4 h; the gel was neutralized in 1.5 M NaCl and 0.5 M Tris-HCl, pH 6.8, for 5 h. The blotting was performed essentially as described by Southern (1975). We prehybridized, hybridized, washed, and exposed the filter to film as described by Jeffreys & Flavell (1977a,b).

p167kRI Clone. The cDNA clone used as a hybridization probe was constructed by P. Early in L. Hood's laboratory (unpublished experiments) from MOPC-167 κ mRNA. The 900-bp cDNA insert flanked by *Eco*RI endonuclease sites is oriented so that the 5′ end of the mRNA is toward the *Hin*dIII site of pMB9. The insert includes the entire 3′ untranslated region (excluding the five terminal residues), the C region, the V region, and a portion of the leader sequence (P. Early, unpublished experiments). The insert is not cleaved by any of the six enzymes used to digest genomic DNA for the Southern blots (i.e., *Eco*RI, *Hin*dIII, *Pvu*II, *Kpn*I, *Xba*I, and *Bam*HI) (J. Miller, unpublished experiments). The insert is cleaved twice by *Hin*cII to yield a 490-bp fragment which contains the 3′ untranslated and most of the C region, a 300-bp fragment which contains most of the V region, and a 110-bp fragment which contains overlapping sequences for both the C and V regions including the J sequence (Lenhard-Schuller et al., 1978). The most likely *Hin*cII site in the V region (by protein sequence) lies at amino acid position 93 (P. Early, personal communication).

Preparation of V- and C-Region Fragments. DNA from p167kRI was cleaved with *Hin*cII and *Eco*RI and electrophoresed a 5% aqueous polyacrylamide gel (Maniatis et al., 1975). Following visualization with ethidium bromide, the 300-bp V-region and 490-bp C-region fragments were excised and electrophoretically eluted. The eluate was passed twice over a hydroxylapatite (HAP) column (Jeffreys & Flavell, 1977a), which was subsequently washed with 0.12 M phosphate buffer (pH 6.8) at room temperature and then again at 60 °C. The DNA was eluted with 0.3 M phosphate buffer (pH 6.8) at 60 °C, and the phosphate was removed by passage over Sephadex G-75. Fractions containing DNA were pooled and ethanol precipitated.

Labeling of p167kRI by Nick Translation. The procedure used was based on that of Rigby et al. (1977). Two hundred pmol of [α-^{32}P]dCTP (Amersham Corp., 2000–3000 Ci/mmol) was dried in vacuo. To this was added 1.0 μg of DNA, 200 pmol of dATP, 200 pmol of TTP, and 1000 pmol of dGTP in 40 μL of glass-distilled H_2O and 50 μL of NTB [100 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 20 mM 2-mercaptoethanol, and 100 μg/mL BSA (Sigma, crystallized and lyophilized)]. Ten microliter of DNase (Worthington) was added to yield a final concentration of 100 ng/mL and a final volume of 100 μL. This reaction mix was incubated at room temperature for 1 min prior to the addition of 6–12 units of *Escherichia coli* DNA polymerase I (Worthington). The final mixture was incubated at 15 °C for 90 min. The reaction was stopped by the addition of 100 μL of 0.5 M EDTA and heated to 65 °C for 10 min. The reaction mixture was chromatographed on Sephadex G-75 into 10 mM Tris-HCl, pH 8, 10 mM NaCl, and 2 mM EDTA. The specific activity of the pooled fractions ranged from 3×10^7 to 10^8 cpm/μg. Reactions which contained less than 1.0 μg of DNA were proportionally reduced in volume.

Results

MOPC-167 Restriction Enzyme Analysis and Mapping. MOPC-167 DNA was cleaved with the six restriction enzymes singly and in all possible pairs. The digests were electro-

[1] Abbreviations used: C_κ gene, gene coding for the constant region of κ chains; V_{167}, gene coding for the variable region of the MOPC-167 κ chain; UT, untranslated region of mRNA.

Table I: Sizes of Bands Detected in Blots of MOPC-167 DNA[a]

	Kpn	*Eco*	*Pvu*	*Xba*	*Hin*d	*Bam*
			Purified C-Region Probe			
alone		~30	18.5	6.1	4.9	5.0
Eco	~23					
Pvu	18.5	17.5				
Xba	6.1	6.1	6.1			
*Hin*d	4.9	4.9	4.9	4.9		
Bam	5.0	5.0	5.0	3.9	2.8	
			Purified V-Region Probe			
alone		~30	18.5	3.4	7.3	5.0
Eco	~23					
Pvu	18.5	17.5				
Xba	3.4	3.4	1.3			
*Hin*d	6.1	7.3	2.2	3.4		
Bam	5.0	5.0	5.0	1.1	1.9	

[a] The sizes in kilobase pairs of bands detected in Southern transfer hybridizations (shown in Figure 1) are tabulated here. These sizing measurements were made by comparing the migration of the band detected by autoradiography to the migration of *Eco*RI-cleaved λ phage DNA detected by ethidium bromide fluorescence.

phoresed on 0.6% agarose gels which subsequently were subjected to Southern's blotting technique (Southern, 1975). Purified V_{167} and C_κ probes, derived from the p167kRI clone, were used in the hybridization analysis. The resulting autoradiograms are shown in Figure 1, and the sizes of the fragments detected are tabulated in Table I. Since purified V- and C-region probes were used and since all digestions gave rise to only one major hybridization band, bands could unambiguously be determined to contain V and/or C regions. The construction of the restriction map was therefore straightforward (Figure 2b).

Analysis of the V- and C-region maps revealed that the C-region- and V-region-containing fragments could be placed in close proximity to one another. Both probes hybridized to a 30-kb band in the *Eco*RI digest, an 18.5-kb band in the *Pvu*II digest, and a 5.0-kb band in the *Bam*HI digest. In the *Bam–Xba* double digest, the C-region-containing band (3.9 kb) and the V-region-containing band (1.1 kb) taken together add up to the size of the *Bam* alone fragment (5.0 kb). A similar result is obtained when comparing the size of the *Bam–Hin*d fragments (2.8 kb for C and 1.9 kb for V) to that of the *Bam* alone fragment, except here the double-digest fragments fall short in length by 0.3 kb. This could either be a sizing error or it could indicate the existence of two *Hin*dIII sites within the 5.0-kb *Bam* fragment, as shown in Figure 2b. It is clear that the 5.0-kb band seen in the *Bam* alone digest represents a single 5.0-kb fragment, containing both the V and C regions; it is not a case of two comigrating 5.0-kb fragments.

The two separately derived C- and V-region maps also show that certain restriction enzyme sites on one map correspond to sites found on the other map. In Figure 2b, these sites are doubly labeled above and below the line representing the DNA. The V- and C-region-containing fragments therefore are only ~1 kb apart. That predicts the insertion sequence between the V and the C region to be between 1 and 4 kb, the size uncertainty resulting from the unknown location of the C region within the C-region-containing fragment.

"Germline" Tissue DNA Restriction Analysis and Mapping. DNAs from liver, Krebs ascites cells, spleen, and thymus were cleaved with the six restriction endonucleases singly and in all possible pairs. Digested DNAs were then subjected to hybridization analysis using the Southern blotting technique. Three probes were used: the V + C probe (i.e., full p167kRI clone), a purified V-region probe, and a purified C-region probe. The autoradiograms of the V + C blot for single-

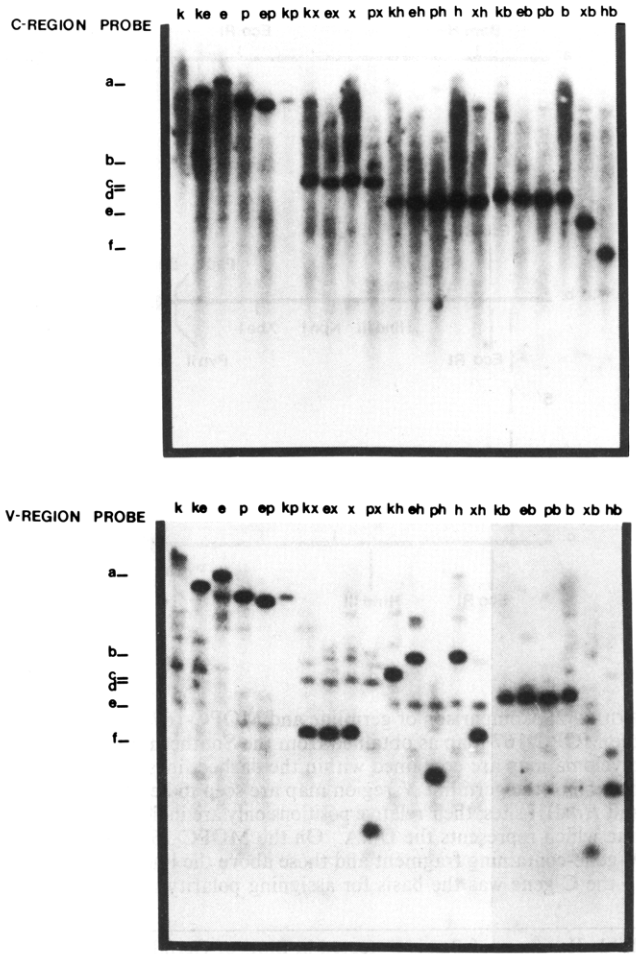

FIGURE 1: Autoradiograms of Southern blots of MOPC-167 DNA using purified V- and C-region probes. MOPC-167 DNA was cleaved with the enzymes and enzyme pairs indicated, electrophoresed on 0.6% agarose gels, and subjected to Southern blot analysis using purified V- and C-region probes. The positions of *Eco*RI-cleaved λ DNA fragments, run in the same gel, are indicated by the bars (a = 21.7 kb, b = 7.5 kb, c = 5.8 kb, d = 5.6 kb, e = 4.9 kb, and f = 3.5 kb). The molecular weights of the hybridization bands are tabulated in Table I. Enzymes are indicated by the first letter of their name.

enzyme digests are shown in Figure 3. The autoradiograms of Figure 3 also show data for myeloma DNAs analyzed with the V + C probe. These data are discussed below. The purified V- and C-region probe blot autoradiograms of single-enzyme digests (data not shown) and the V + C probe blot autoradiograms of double-enzyme digests (not shown) enabled us to construct restriction maps for the V- and the C-region genes and their flanking sequences. The normal tissue DNAs (liver, spleen, and thymus), as well as Krebs ascites DNA, always exhibit only one or two major bands with the V + C probe and only one major band with purified V- or C-region probes. This was true for all single- and double-enzyme digests. It was therefore possible to unambiguously assign bands to the V or the C region. The sizes of these germline tissue bands, single and double digests, are shown in Table II. In all cases, the banding pattern of Krebs DNA was identical with normal tissue DNA. The maps resulting from these data are shown with the MOPC-167 map in Figure 2 (parts a and c). The C-region and V-region germline tissue maps cannot be joined together as in the MOPC-167 map.

Restriction Enzyme Analysis of Other Myeloma DNAs. DNAs from κ-producing myelomas MOPC-41, MOPC-21 and MOPC-321 and λ-producing myeloma S178 were singly and doubly digested with all six restriction enzymes and all fifteen

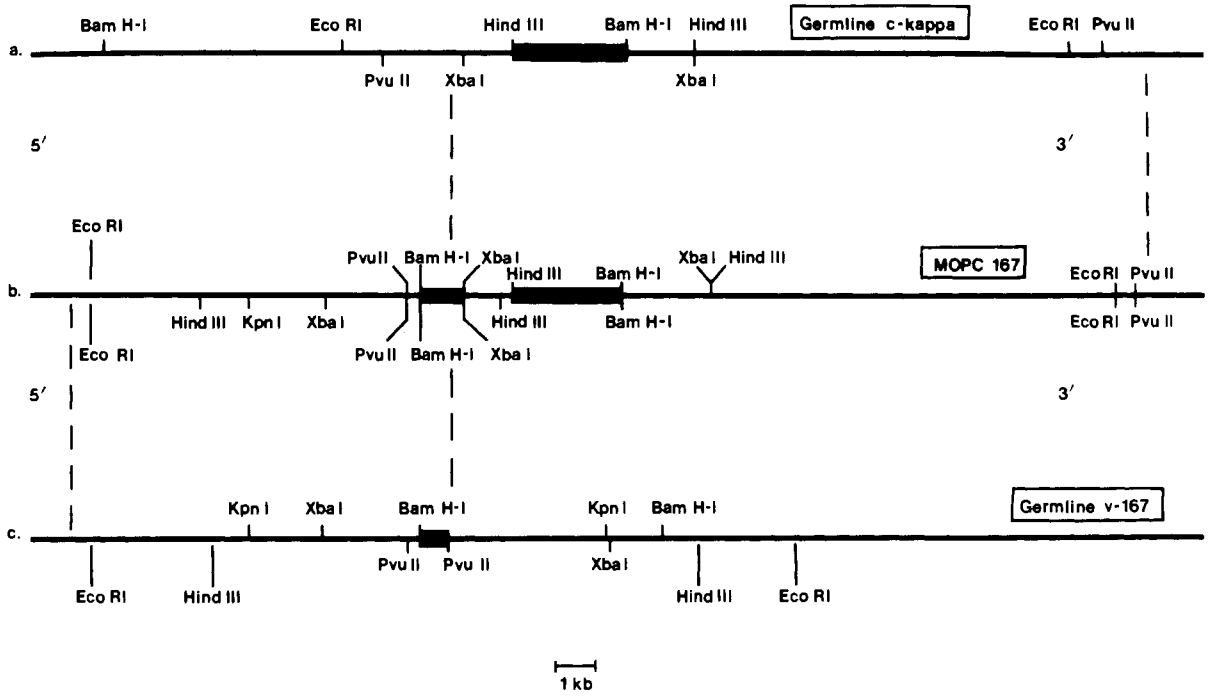

FIGURE 2: Comparison of germline and MOPC-167 restriction maps. The germline C- (a) and V-region (c) maps are illustrated here beside the MOPC-167 map as obtained from the Southern blots shown in Figure 1. The regions of the germline maps which are identical with the myeloma map are contained within the dashed lines connecting the respective maps; the 3′ portion of the germline C-region map and the 5′ portion of the germline V-region map are seen to be identical with the MOPC-167 map. It is not possible to accurately place certain *Eco*RI and *Hind*III sites; their relative positions only are indicated, and they are illustrated by means of a long line, not connected to the main horizontal line which represents the DNA. On the MOPC-167 map, restriction enzyme sites illustrated below the line were obtained by mapping the V-gene-containing fragment and those above the line by mapping the C-gene fragment. The location of V_{167} in the MOPC-167 map relative to the C gene was the basis for assigning polarity in the germline maps.

Table II: Sizes of Bands Detected in Blots of Germline DNA[a]

	Kpn	*Eco*	*Pvu*	*Xba*	*Hind*	*Bam*
		C-Region Bands				
alone	~30	18	17	5.6	4.0	12.9
Eco	18					
Pvu	17	17				
Xba	5.6	5.6	5.6			
Hind	4.0	4.0	4.0	4.0		
Bam	12.9	7.1	6.1	4.0	2.9	
		V-Region Bands				
alone	8.8	18	1.3	7.1	9.8	6.5
Eco	8.8					
Pvu	1.3	1.3				
Xba	7.1	7.1	1.3			
Hind	9.8	9.8	1.3	7.1		
Bam	4.6	6.5	1.0	4.7	6.5	

[a] The sizes in kilobase pairs of normal tissue DNA detected in Southern transfer hybridizations (shown in part in Figure 3) are tabulated here. The sizing measurements were performed as described in Table I.

enzyme pairs. Single-enzyme digests were analyzed on Southern blots using the V + C probe (see Figure 3) and the purified C- and V-region probes (purified C- and V-region blots not shown). Double-enzyme digests were analyzed with the V + C probe (blots not shown). The sizes of the bands detected in all of these experiments are tabulated in Table III.

All myelomas possessed a V-region map that was identical with that of the normal tissue DNA (see Figure 2c). The molecular weight data are essentially the same as those shown in Table II.

The blot data made it obvious that all myelomas had two or more C-region maps, and except for MOPC-321, which showed a germline C-region map in addition to two rearranged C-region maps, all C regions differed from the germline C region. The restriction maps are shown in Figure 4. The precision with which a restriction site is placed on the map deteriorated as its map position increased from the C-region-containing fragment. In constructing the maps, it was usually simple to determine which set of fragments corresponded to a given C region. In a few more complex cases, shown in Figure 4 (parts g, h, and k), maps could be constructed but there were some minor ambiguities, not further discussed here.

Discussion

The extent of rearrangements of V_{167} and C_κ genes in different cell types was investigated by restriction mapping. The six hexanucleotide specific restriction endonucleases used in this study do not cut within the V, C, or UT sequences of MOPC-167 used as a radiolabled probe (see Experimental Procedures). Therefore, the finding of more than one radiolabeled restriction fragment in the Southern blots means that portions of the DNA sequence coding for the mature MOPC-167 mRNA are physically separated in the genome. This is the case in all cell types, as the V_{167} and the C_κ sequences appear in different fragments with several of the restriction enzymes tested (Figures 1 and 3). This finding is in agreement with other studies which have shown that V and C genes are not contiguous regardless of whether or not they are expressed (Lenhard-Schuller et al., 1978; Seidman & Leder, 1978).

It has been shown by others (Tonegawa et al., 1977) that immunoglobulin genes in nonlymphoid cells (kidney and liver) are not rearranged, i.e., are found in the same context as in embryo DNA which seems to be the germline context. In the present study, we have referred to the patterns of hybridization of κ sequences with liver DNA as germline patterns. The present study shows that certain DNA sequences expressed

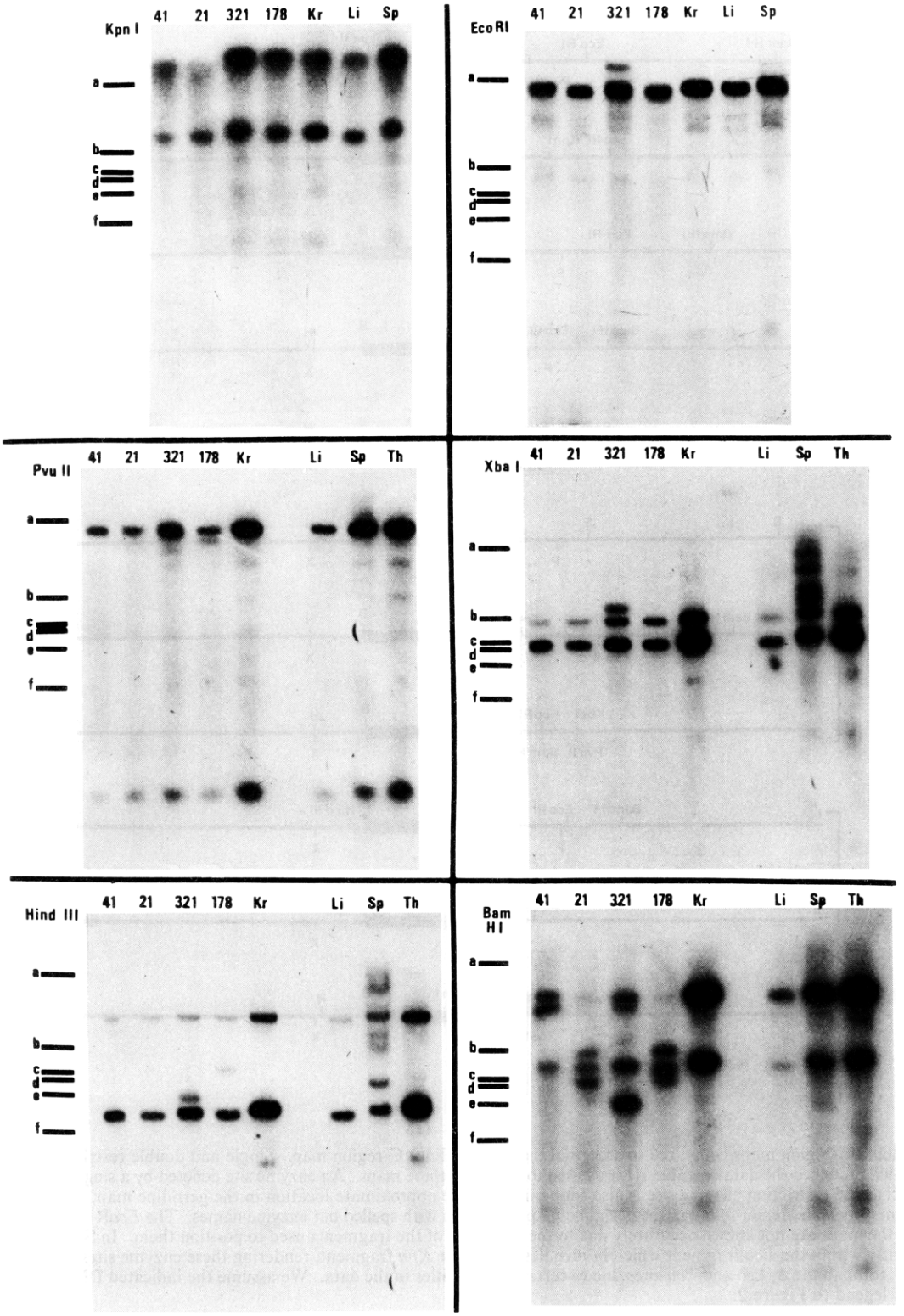

FIGURE 3: Southern transfer hybridizations using a full-length (C + V region) probe with single-enzyme digests. Myeloma DNAs MOPC-41, MOPC-21, MOPC-321, and S178, Krebs ascites DNA, and DNAs from liver, spleen, and thymus were digested with the restriction endonucleases indicated. The digests were electrophoresed in 0.6% agarose gels and then transferred to nitrocellulose sheets (Southern, 1975). A nick-translated cloned κ cDNA probe, p167kRI, was hybridized to the DNA on the nitrocellulose sheets which were then autoradiographed (see Experimental Procedures). The positions of *Eco*RI-cleaved phage λ DNA fragments, run in the same gel, are indicated by the bars (a = 21.7 kb, b = 7.5 kb, c = 5.8 kb, d = 5.6 kb, e = 4.9 kb, and f = 3.5 kb). Abbreviations used: 41, MOPC-41; 21, MOPC-21; 321, MOPC-321; 178, S178; Kr, Krebs ascites; Li, Liver; Sp, spleen; and Th, thymus. The molecular weights of the hybridization bands are tabulated in Tables II and III. The several high molecular weight fragments seen with spleen DNA digested by *Xba*I and *Hin*dIII are due to incomplete digestion of this DNA, as observed in the EtBr-stained gels (not shown). When these digestions of spleen DNA were repeated, only germline bands were obtained (Selsing, unpublished experiments).

in the κ chains of the MOPC-167 myeloma are found in a nongermline context in all five myelomas investigated. Closer inspection with a V-specific probe indicates that V sequences are always in a germline context in four of the myelomas and in the other tissues studied. None of the four myelomas express V_{167}. This indicates that expression of other specific

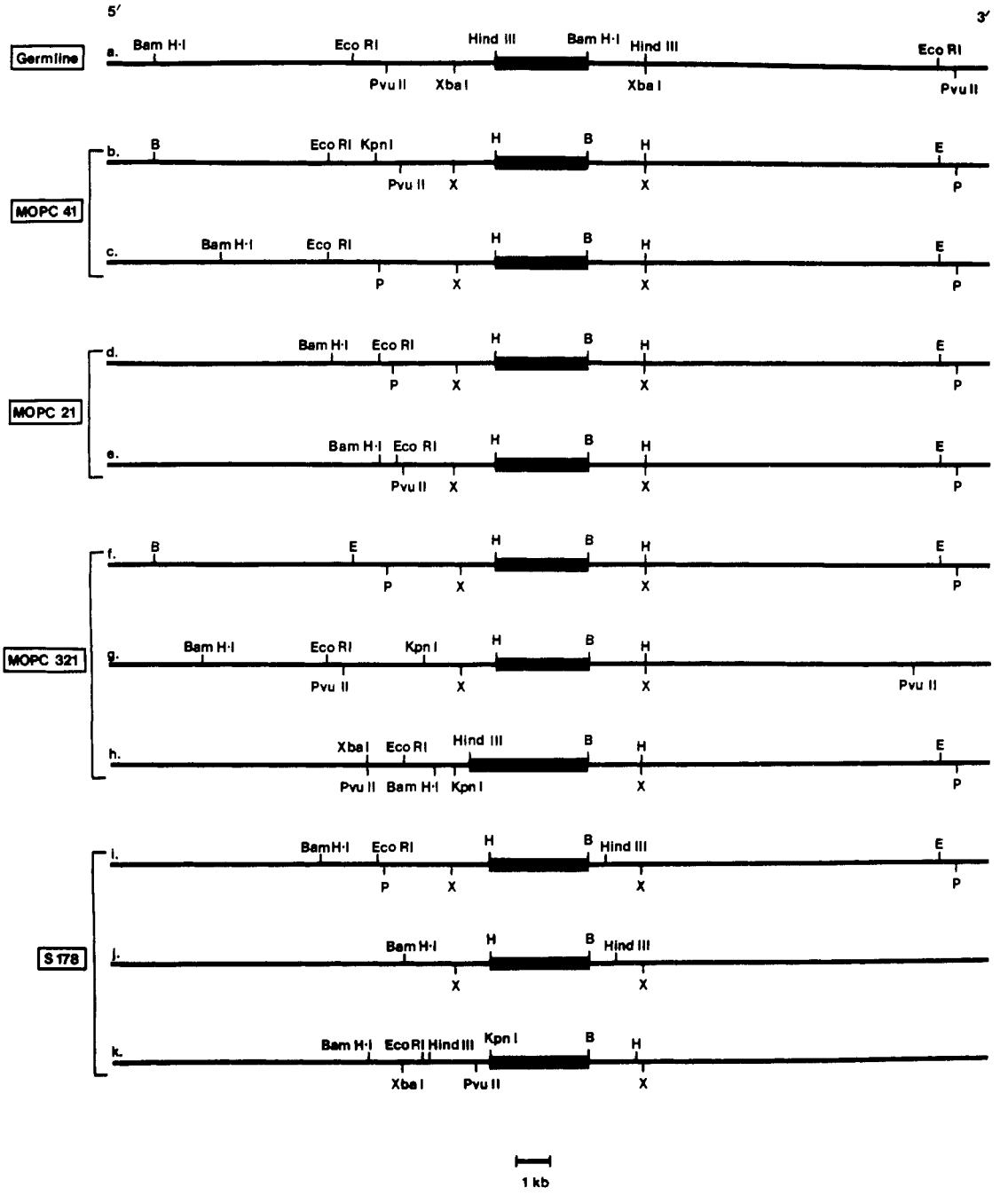

FIGURE 4: Myeloma C-region maps compared to a normal tissue (germline) C-region map. Single and double restriction enzyme Southern blot analysis, which produced the data of Table III, was used to construct these maps. An enzyme site denoted by a single letter in the myeloma maps indicates that the restriction enzyme site is also found in the same approximate location in the germline map; restriction enzyme sites in the myeloma maps which are not found in the germline map are shown with spelled out enzyme names. The *Eco*RI and *Pvu*II site positions at the 3′ end on the maps are not known accurately due to the large size of the fragments used to position them. In S178 map j, the two *Pvu* sites are apparently within the *Eco* fragment which in turn lies within the *Kpn* fragment, rendering these enzyme sites unmappable. In S178 map k, we could not map the 3′ *Eco* and *Pvu* sites due to certain ambiguities in the data. We assume the indicated DNA polarity for reasons presented in the legend to Figure 2.

V regions in these myelomas does not alter the context of V_{167} sequences. The V_{167} gene is, however, rearranged in the MOPC-167 myeloma.

Besides the prominent V-region bands, weak bands are seen in the hybridization with the total MOPC-167 clone and the V probe (Figures 1 and 3). We believe that they correspond to V genes which are closely related to V_{167}, i.e., belong to the same κ subgroup. Seidman et al. (1978a,b) have shown that V genes which appear to belong to the same subgroup cross-hybridize in Southern blots but that the radioactive signal is weaker than with the identical sequence. The multiple V_{167}-related sequences appear to be in the same restriction fragments in all DNAs tested, suggesting that they are germline sequences.

Rearrangements of C_κ sequences were observed in all myelomas tested. Only one myeloma, MOPC-321, showed a germline map of one C_κ sequence (Figure 4). In all five myelomas we have found one to three C_κ genes in a rearranged context. The data on MOPC-41 disagree with a previous report (Seidman & Leder, 1978) of a germline C_κ region in this tumor. We also observe an apparent germline sequence when MOPC-41 DNA is analyzed by individual enzymes only; however, when double-enzyme digestions are performed, it is obvious that MOPC-41 DNA has two rearranged C_κ genes

Table III: Tabulation of Sizes of C-Region Hybridization Bands from Four Myelomas[a]

enzyme(s)	DNA source	size of band[b] (kb)	map[c]
Kpn	all	~30	b–k
Eco	all	18	b–f, h–k
	321	~30	g
Pvu	all	17	b–k
Xba	all	5.6	b–g, i, j
	321	8.1	h
	178	7.1	k
Hind	all	4.5	b–g, i, j
	321	4.8	h
	178	6.2	k
Bam	41	12.9	b
		11.1	c
	21	7.3	d
		5.5	e
	321	12.9	f
		11.4	g
		4.6	h
	178	7.3	i
		6.2	k
		5.5	j

enzyme(s)	DNA source	size of band[b] (kb)	enz 1[c]	enz 2[c]	pair[c]
Kpn, Eco	all	17		c–f, i	b, g
	321	14			h
	178	11.5			k
Kpn, Pvu	all	17		b–f, i	g
	321	15.5			h
	178	16.0			k
Kpn, Xba	all	5.6		b–g, i, j	h
	178	4.6			k
Kpn, Hind	all	4.6		b–g, i, j	k
	321	5.6		h	
Kpn, Bam	41	11.3		c	
		6.3			b
	21	7.9		d	
		6.0		e	
	321	12.9		f	
		4.9			g
		4.0			h
	178	7.9		i	
		6.0		j	
		3.0			k

enzyme(s)	DNA source	size of band[b] (kb)	enz 1[c]	enz 2[c]	pair[c]
Eco, Pvu	all	17.0	h	g	b–f, i
	178	16.0			k
Eco, Xba	all	5.6		b–g, i, j	
	321	7.1			h
	178	7.1			k
Eco, Hind	all	4.5		b–g, i, j	
	321	5.2		h	
	178	6.9		k	
Eco, Bam	41	7.6			b, c
	21	6.3			d, e
	321	7.8			f, g
		4.8		h	
	178	6.2			i
		5.0		j	k
Pvu, Xba	all	5.6		b–g, i, j	
	321	8.6		h	
	178	5.0			k
Pvu, Hind	all	4.5		b–g, i, j	
	321	5.2		h	
	178	5.1			k
Pvu, Bam	41	6.3			c
		5.7			b
	21	5.9			d
		5.5			e
	321	7.3			g
		6.1			f
		4.7		h	
	178	6.0			i
		5.7		j	
		3.4			k
Xba, Hind	all	4.6		b–g, i	
	321	5.2		h	
	178	6.8		k	
Xba, Bam	all	4.0			b–j
	178	5.5			k
Hind, Bam	all	2.9			b–g, i, j
	321	3.6			h
	178	5.2			k

[a] MOPC-41, MOPC-21, MOPC-321, and S178. [b] The sizes in kilobase pairs of bands detected in Southern transfer hybridization shown in part in Figure 3. The sizing measurements were made as described in footnote *a* of Table I. [c] Letters in these columns refer to the maps shown in Figure 4. The subheadings "enz 1", "enz 2", and "pair" indicate which of the two enzymes or whether both enzymes produced the fragment which ultimately appeared in the maps indicated in these columns.

(Figure 4). Thus, MOPC-321 is the only myeloma we studied which appears to have a germline C_κ sequence. In addition, it has two rearranged C_κ sequences; i.e., three different C_κ genes are observed in this myeloma. It is unlikely that the germline sequence is due to host cells which infiltrated the tumor since the amount of infiltrating cells is only ~10–20% (P. Ralph, personal communication) and since four other myelomas do not show this pattern. The MOPC-321 cell may be triploid for the chromosome carrying κ genes, as all myelomas are polyploid. Alternatively, this particular myeloma or possibly all cells may have more than one C_κ gene per haploid set. Recent DNA saturation hybridization data indicate two C_κ genes per haploid genome (Valbuena et al., 1978). If this were the case, the one to three new C_κ patterns seen with the other myelomas may be due to identical rearrangements of two or more C_κ genes. Clearly, this question requires further study.

The observation that each myeloma cell except MOPC-167 has two or three C_κ genes in a rearranged context has to be reconciled with the fact that phenotypically these cells show allelic exclusion, i.e., produce only one type of κ chain. It remains to be determined at what stage in the synthesis of immunoglobulin mRNAs allelic exclusion takes place. But one also has to keep in mind that these myeloma cells are heteropolyploid and possibly multiclonal (see below).

It was unexpected to find rearrangement of three C_κ genes and no germline C_κ in S178, a myeloma which produces λ chains. Apparently, λ-producing myelomas exist which show no rearrangement of C_κ genes (Lenhard-Schuller et al., 1978). The S178 myeloma contains κ RNA, but ~20 times less than λ RNA (Storb et al., 1977). These cells require, therefore, a mechanism for differential control of the quantities of κ and λ mRNA synthesis. The fact that the S178 myeloma has no C_κ gene in a germline context suggests that the simultaneous synthesis of λ and κ RNA is not the result of fusion of two different cells.

On the background of the extensive translocations observed with myeloma cells, it appears striking that spleen and thymus DNA only showed the germline pattern. It was previously found by in situ hybridization that 87% of spleen cells and 99% of thymus cells contain κ RNA (Storb, 1978). Thus, essentially all B and T cells express a κ gene in the mouse where over 95% of the immunoglobulin L chains are κ. The thymus DNA used in the present study was prepared from single

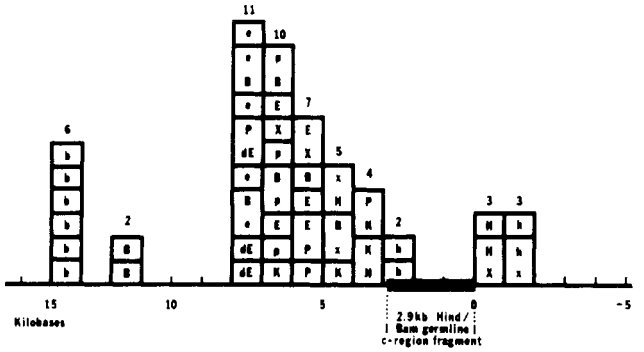

FIGURE 5: Tabulation of restriction site changes in myeloma C-region maps compared to the germline C-region map. The C-region maps obtained from the myelomas were systematically compared to the germline map over 1-kb intervals. If a restriction site was present in a given interval in the germline C_κ map and lacking in the myeloma map, this alteration is notated in the figure by a lower case letter which is an abbreviation of the enzyme involved. Similarly, if a site appeared in the myeloma map which was absent in the germline map, this alteration is denoted by an upper case letter. Enzyme site shifts within a given interval are denoted by prefixing the enzyme abbreviation with a "d". If a restriction site shift occurred, it was considered to be significant if it was within 5 kb of the smallest hybridizing fragment (the *Hin*dIII–*Bam*HI fragment in most cases) and if the shift itself was greater than 0.5 kb. When shifts occurred more than 5 kb away from the C-region-containing fragment, they were considered to be relevant only if they were greater than 1 kb. Alterations which occurred within the same myeloma map in a given 1-kb interval are delineated by boxing them together in the figure. *Eco*RI and *Pvu*II sites which often appeared 15 kb to the right of the C region were omitted from this analysis because their positioning is subject to too much error. Abbreviations: B or b, *Bam*HI; E or e, *Eco*RI; H or h, *Hin*dIII; K or k, *Kpn*I; P or p, *Pvu*II; and X or x, *Xba*I.

thymus cells (99% T cells), thus eliminating fibroblasts, etc. Since all myeloma cells studied showed rearranged C_κ genes and generally no germline gene, one would have expected to find in thymus and spleen cells either a very mixed rearrangement pattern or no specific C_κ hybridization bands at all because of the complexity of the κ gene repertoire which could be expressed. If thymus and spleen B and T cells had one germline and one rearranged C_κ gene, one would possibly obtain the observed germline pattern in Southern blots because of lack of overlap on the gel of restriction fragments carrying rearranged genes. Thus, the question remains why spleen and thymus cells would present nonrearranged germline genes although these seem to be absent in three out of four myelomas.

The lack of a germline C_κ gene in myelomas and not in thymus and spleen may be related to the malignancy or the differentiated plasma cell status of the former. They are heteropolyploid and may have lost a chromosome carrying a germline C_κ gene. It seems unlikely that immunoglobulin gene rearrangement occurs only in malignant cells. Firstly, a control tumor, Krebs ascites, does not possess rearranged C_κ genes (Figures 2 and 3). Secondly, V_{167} genes were not rearranged in any of the four myelomas which do not express V_{167}, a significant internal control. Also, the C_λ gene is not rearranged in the four κ myelomas studies (R. Wilson, J. Miller, and U. Storb, unpublished experiments). Furthermore, we have not found any globin gene rearrangement in the four myeloma cells studied in analysis with *Eco*RI, *Bam*HI, *Pvu*II, and *Hin*dIII (J. Miller, unpublished experiments). Lastly, and most importantly, myeloma cells behave like normal plasma cells in all parameters related to immunoglobulin synthesis, and the fine structure of the immunoglobulin gene translocations studied in cloned sequences suggests a functional rearrangement (Brack & Tonegawa, 1977; Brack et al., 1978; Lenhard-Schuller et al., 1978; Seidman & Leder, 1978; and this report Figures 1 and 2). However, it remains to be tested directly in a homogeneous cell population whether normal lymphoid cells rearrange C_κ genes and, if so, at which stage of their development.

The restriction maps of the germline and rearranged C_κ genes have all been drawn so that the *Bam*HI site is on the right side of the smallest C_κ positive fragment (Figures 2, 4, and 5). When one compares the MOPC-167 C_κ map with the germline and other myeloma C_κ maps (Figures 2 and 4), it becomes apparent that the 5′ end of the sequence coding for κ mRNA and the V region would be to the left of C in all the maps. In the MOPC-167 myeloma a complete map of the joined V and C genes and flanking sequences was obtained from the overlapping V and C maps (Figure 2). There is a noncoding region of ~1 kb between the smallest fragments containing the V and C genes. This supports the findings by others (Lenhard-Schuller et al., 1978; Seidman & Leder, 1978) of an intron between the V_κ and C_κ genes. The sequence at the 5′ side of the V_{167} gene in MOPC-167 is identical with the sequence adjacent to one end of the V_{167} germline gene found in myelomas which do not express V_{167} and in other tissues. Thus, if V and C genes are joined by V gene translocation, which is one of several possible alternatives of the joining mechanism, the V gene must carry a considerable amount of flanking DNA with it. The 3′ end of the V_{167} gene containing fragment in MOPC-167 differs from that in the germline V_{167} gene (Figure 2). The joining occurs therefore somewhere within the small *Bam–Pvu* fragment which carries the V_{167} germline sequence (Figure 2c).

In the other myelomas, the major changes of restriction sites occur beyond the 5′ end of the C gene (Figures 4 and 5). As the data of others have shown, the total complex of an expressed κ gene of V, J, intervening sequence, C, and 3′-UT comprises ~3.7 kb (Lenhard-Schuller et al., 1978; Seidman & Leder, 1978). Thus, the portion of DNA with the greatest variability, 4 and 5 kb toward the 5′ end from the C fragment (Figures 5), lies already outside of the V region but within the sequence coding for the mRNA precursor (Gilmore-Hebert et al., 1978). It corresponds to the region flanking the V gene which is also present in the germline arrangement of the same V gene (Figure 2). Apparently, subgroup-specific variations in the sequences surrounding different V genes vary greatly.

Only three C_κ maps show changes beyond the 3′ end of the C_κ gene which may be significantly different from the germline map. They are sequence g of MOPC-321 (Figure 4) and sequences i and j of S178 (Figure 4). The former lacks the *Eco*RI site present at 10 kb from the C fragment in the other C_κ maps. The latter two have moved the *Hin*d site closer to the C fragment. The rearrangements due to V gene joining are most likely to occur to the left of the C_κ fragment in these maps (see above). Thus, the changes to the right are suggestive of the existence of multiple different C_κ genes. At this point, however, the possibility cannot be excluded that V joining occurs by the insertion of the C gene into a V gene pool. In that case, alterations beyond the 3′ end of C could be due to variations in the insertion site. In any case, the question requires further study with more detailed restriction mapping and cloning.

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Internal Microviscosity of Red Blood Cells and Hemoglobin-Free Resealed Ghosts: A Spin-Label Study†

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ABSTRACT: Although important for the understanding of intracellular events, the internal viscosity of red blood cells is not known. We present electron spin resonance data which show the effect of cell size, extracellular pH, and temperature on the motion of a small spin-label, 4-amino-2,2,6,6-tetramethylpiperidinyl-*N*-oxy (Tempamine), both in the presence and absence of hemoglobin. Tempamine motion in the interior of intact red blood cells is hindered by a factor of 5.47 ± 2.04 at pH 7.5, 25 °C, and 320 mosm, while in resealed ghosts Tempamine motion is hindered by a factor of 2.34 ± 0.42. Both values decrease with increasing cell size, increase with increasing pH (to pH 9), and decrease with increasing temperature. Further data exclude the possibility that binding of Tempamine to red cell membranes or hemoglobin could account for our results. Tempamine is apparently randomly distributed throughout the red cell interior both in the absence and presence of hemoglobin. We suggest that the aqueous interior of both intact and resealed red blood cells and ghosts hinders the motion of Tempamine. This further suggests that the microviscosity inside the red cell and resealed ghosts is greater than that of bulk water.

The internal viscosity of cells has been shown to be greater than that of bulk water by a number of methods, such as nuclear and proton magnetic resonance [see Cooke & Kuntz (1974) and Hazlewood (1973) for reviews] and more recently by electron spin resonance (Keith & Snipes, 1974; Morse et al., 1975; Henry et al., 1976; Haak et al., 1976; Keith et al., 1977a; Morse, 1977; Berg et al.,1979). The results obtained by these techniques show that water diffusion and spin-label rotation are hindered inside cells and organelles by factors of 2–40 compared to bulk water (depending on the method). The source of this increased intracellular viscosity is unknown.

Recent evidence suggests that the cell membrane may contribute in some way to the intracellular viscosity. Morse et al. (1975) found that intravesicular viscosity of lobster sarcoplasmic reticular vesicles was ~20–40 times greater than that of bulk water. This was not caused by proteins in the internal aqueous space, and they concluded that the inner surface of the membrane was influencing the order of the internal aqueous environment. Further studies by Berg et al. (1979) on spinach thylakoids suggested that the membrane-associated proteins influence the apparent viscosity of intracellular water.

A previous study by Dintenfass (1968) suggests that maintenance of the internal viscosity of the red blood cell at some nominal value is required for proper circulatory function. The values for internal viscosity obtained by Dintenfass are,

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