Volume 8 Number 20 1980 Nucleic Acids Research

Comparison of different rearranged immunoglobulin kappa genes of a myeloma by electronmicroscopy and restriction mapping of cloned DNA: implications for "allelic exclusion"

Alan M.Walfield, Ursula Storb**, Erik Selsing and Hanswalter Zentgraf*

Department of Microbiology and Immunology, University of Washington, Seattle, WA 98195, USA, and *Deutsches Krebsforschungszentrum, Heidelberg, GFR

Received 21 July 1980

ABSTRACT

We have studied the organization and function of different rearranged kappa genes in a myeloma, MOPC-21. Two kappa genes were cloned into Charon 4A and compared with each other and with a cloned germline C_κ gene by restriction mapping and electron microscopy. One MOPC-21 clone corresponds to the gene coding for the MOPC-21 kappa chain polypeptide; it has the V_{21} gene joined with the C_κ gene at the J2 sequence. The other MOPC-21 clone corresponds to a nonfunctional rearranged MOPC-21 kappa gene, except for a 1kb deletion, 3' of J4. A similar deletion is also found in a "new" kappa gene present in NS-1, a cellular subclone of MOPC-21. The clone of the "nonfunctional" kappa gene has a V gene which is distinct from V_{21} which is joined to C_κ in the vicinity of J2. The undeleted form of this gene codes for a κRNA having the size of mature κmRNA which, however, is not translated into kappa chains. Thus the defect of the "nonfunctional" gene manifests itself at a late step of gene expression. The basis for "allelic exclusion" of antibody genes may simply be the complexity of the processes between genes and gene products, resulting in the expression of only one gene.

INTRODUCTION

It is now widely accepted that the genes coding for antibodies have somatically recombined V(1) and C gene segments, separate in germ line tissue, into a contiguous functional unit (2-4). The exact relationship of V-C somatic recombination to gene expression is however not well understood, particularly in the light of the multiple C_κ gene rearrangements found in monoclonal myelomas (5-7). It appears that in these cases only one of the rearranged genes, the "functional" gene, actually codes for kappa chains (8). An understanding of the defects in rearranged "nonfunctional" kappa genes that prevent expression on the protein level will aid in elucidating the

control of expression of functional kappa genes.

The present study deals with the differences of "functional" and "nonfunctional" kappa genes in the myeloma MOPC-21 and its derivatives, NS-1 and NS-1n. MOPC-21 has two rearranged C_K genes which after digestion with the restriction enzyme Bam H1 give rise to two C_K fragments of 6.9 and 5.2kb (Ref. 9-note that the fragment sizes have been slightly revised). The subclone NS-1 has the same two, together with a third rearranged gene (5.9kb BamH-1 fragment) which is likely to have arisen from a minor subpopulation of MOPC-21 cells (Ref. 8 and see Results). NS-1n is a variant of NS-1 which has lost the 5.2kb Bam gene and concomittantly the synthesis of the MOPC-21 or NS-1 kappa protein chain (8). Since NS-1n synthesizes no kappa chains, the two remaining C_K genes (Bam 6.9 and 5.9) are apparently "nonfunctional".

We have cloned two different rearranged C_K genes from MOPC-21 to compare their structure. This article describes the electronmicroscopic analysis and restriction mapping of these two cloned DNAs representing a "functional" and a "nonfunctional" gene.

MATERIALS AND METHODS

Strains for cloning. Charon 4A was supplied by F.R. Blattner, as were the host strains E.coli K802, DP50/supF, and CSH18, and the strains for in vitro packaging W3350 and N100.

Preparation of Charon 4A DNA. Charon 4A and Ch.4A recombinants were grown on E.coli DP50/supF by the liquid lysate method of Blattner et al. (10). Ch.4A DNA was prepared by the procedure of Maniatis et al. (11). The outer arms of the phage DNA were prepared by digestion with Eco R1, annealing, and two successive sedimentations through 10-40% linear sucrose gradients (at 25,000 rpm for 24 hr at 4°C).

Preparation of mouse myeloma and spleen DNAs for cloning. MOPC-21 DNA was prepared from serially passaged myelomas and spleen DNA from Balb/c mice as previously described (6). The DNAs were purified of low molecular weight materials by equilibrium sedimentation in CsCl gradients. The DNAs were digested to completion with Eco R1 as described (6) and treated according to the procedure of Maniatis et al. (11) to obtain, after sedimentation through sucrose gradients, a distribution of Eco R1 fragments ranging in size from 16-19kb.

Preparation and selection of recombinant phages. The 16-19kb MOPC-21 DNA fragments were used to create a partial library of mouse Eco R1 fragments. The Eco R1 MOPC-21 fragments were ligated to annealed outer arms

of Ch.4A through the enzyme generated complementary ends in a 1:1 molar ratio according to the method of Blattner et al. (10). The recombinant DNA was packaged in vitro and infected into E.coli K802 (12). The resultant plaques were screened in 150 mm Petri dishes by the plaque hybridization method of Benton and Davis (13). The nitrocellulose filters were hybridized to nick translated cloned cDNA p167kR1 (14), to detect recombinant phages which bear kappa genes. The phage were replated and rescreened until pure. The methods of nick translation for ^{32}P labeling (15), hybridization to the filters and wash conditions were as described (6).

cDNA plasmids. Purified MOPC-21 light chain mRNA was used to prepare ds cDNA essentially as described by Wickens et al. (16). The ds cDNA was tailed with dC residues and mixed with pBR322 (17) which had been linearized using PstI and tailed with dG residues (18). The mixture was used to transform X1776 (19). Transformed clones were selected for tetracycline resistance and screened for ampicillin resistance. Ampicillin sensitive candidate clones were assayed for C_κ sequences by colony hybridization (20) using a C_κ probe obtained from p167κRI. Plasmid size in C_κ positive candidates was ascertained by agarose gel electrophoresis of small lysates (21). Since no size selection of the original ds cDNA was performed, most inserts were found to be 200-300 bp in length. However, one recombinant plasmid, pES111, contained an insert 450 bp in length, and subsequent restriction enzyme analysis indicated that this insert spanned both C_κ and V_{21} sequences. DNA sequence analysis (22) demonstrated that 191 bp of the pES111 insert translated exactly to positions 61-124 of the MOPC-21 light chain amino acid sequence (23). Double digestion of pES111 with Pst I and Alu I allowed isolation of a restriction fragment containing 100 bp of V_{21} sequence exclusive of J2 and C_κ sequences.

The plasmid p167κRI was constructed by P. Early in L. Hood's laboratory (14) from MOPC-167 κmRNA. Preparation of a C region fragment has been described (6).

Recombinant DNA safety. All procedures for the cloning and propagation of recombinant bacteriophages and plasmids were performed in accordance with the NIH guidelines for research involving recombinant DNA molecules (1978) at the P2-EK2 level of physical-biological containment.

Preparation of Kappa mRNAs. Kappa mRNAs were prepared from membrane bound ribosomes of various mouse myelomas as described (24,25).

Heteroduplexes. Heteroduplexes were formed (26) either from total phage DNA clones alone and/or from the Eco R1 inserts which had been separated from

the phage arms by CsCl buoyant density gradient centrifugation. Equimolar amounts (10 µg/ml or 3 µg/ml, respectively) of each DNA were denatured in 0.1N NaOH, 20mM EDTA at room temperature. Tris-HCl (pH 7) and formamide were then added to final concentrations of 100mM and 50% (v/v), respectively. Renaturation was performed at 24°C for 1.5 hr.

R loops. R loops were formed (27) in 0.59M NaCl, 0.084M Hepes pH7.8, 4mM EDTA, 17mM Tris-HCl, pH7, and 59% (v/v) formamide with 10 µg/ml DNA and 23 µg/ml kappa mRNA (unless otherwise indicated in the text). Samples were incubated at 55°C for 12 to 16 hr.

Combination of heteroduplexes and RNA hybrids. MOPC-21 kappa RNA was allowed to hybridize with preformed heteroduplexes by adjusting the heteroduplex mixture to R loop conditions.

Electron microscopy. Electron microscopy was performed as described (28). Briefly, the spreading solution contained 50% formamide, 2M urea, 5mM Tris-HCl, 0.5mM EDTA pH 8.5, 50 µg/ml cytochrome C, 60-200 ng/ml heteroduplexed or R looped DNA, and 30 ng/ml each of a double stranded and single stranded phage DNA size marker (28)(fd=6.4kb or PM.2 = 10kb). The hypophase was double distilled water. Shadowing with Pt/Pd 80/20 was done at an angle of 7°. Electron micrographs were taken with a ZEISS EM 10A at a magnification of 9,200 at 60kV. Prints of electronmicrographs were projected onto a digitizer tablet and the contour lengths of hybridized cloned DNAs and internal DNA standards were determined with the image-analyzing system MOP-AM-01 (Kontron Messgeräte, Eching/München). Calculations and distribution analyses were made with the use of a computer program APL (Institute of Documentation and Statistics, German Cancer Research Center).

RESULTS

Kappa clones. From the partial MOPC 21 gene bank of approximately 2.8×10^5 plaques, four recombinant phage containing C_κ were independently isolated. The clones were of only two different classes, represented by Charon 4A.M21 B_1 and G, respectively. In addition a clone obtained from spleen DNA, Charon 4A.Spl01, is described here.

Correlation of cloned and genomic sequences. In order to correlate the cloned DNAs obtained from MOPC-21 with the genomic kappa sequences, the restriction maps of C_κ gene containing DNA segments were compared (Fig. 1 and Table I). B1 showed the same restriction enzyme sites as the 5.2kb BamH-1 kappa gene of MOPC-21. It also hybridized with the V region sequence of a cDNA clone derived from the kappa mRNA of MOPC-21 (Fig. 6). Clone B1

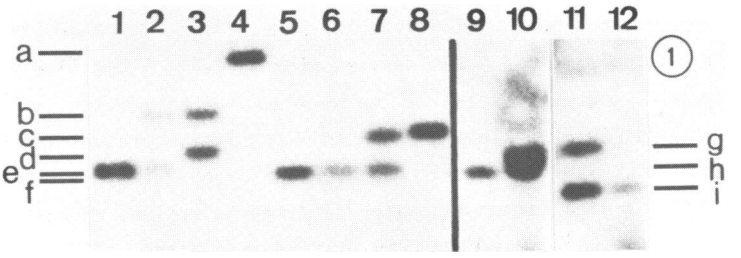


Figure 1. Autoradiogram of Southern blots of myeloma and cloned DNAs. Restriction enzyme digestions were with Bam H1: B1 clone (1), MOPC-21(2), NS-1n (3), G clone (4); with Eco R1/ Xbal: B1 clone (5), MOPC-21 (6), NS-1n (7), G clone (8); with Eco R1/ Bam H1: B1 clone (9), MOPC-21 (10), NS-1n (11), G clone (12). Hybridization probe: cloned C_K cDNA (see Methods). The sizes of cloned DNA fragments were determined in EtBr stained gels with phage lambda Hind III fragments as size markers. The cloned fragments were then used as size markers in the autoradiogram. a=9.5, b=6.9, c=6.4, d=5.8, e=5.2, f=5.1, g=5.9, h=5.2, i=4.7kb.

represents therefore the expressed kappa gene of MOPC-21 (8).

The C_K restriction map of G does not match with either of the two major C_K genes of MOPC-21 (Fig.1 and Table I). It corresponds however to the "new" gene which NS-1 and NS-1n exhibit in addition to the MOPC-21 kappa

Table I. Correlation of Cloned and Genomic Sequences

	Genomic DNAs[a]			Cloned DNAs[a]	
	M 21	NS-1[b]	NS-1n[c]	B_1	G
BAM H-1	6.9	6.9	6.9		
		5.9	5.9		9.5[d]
	5.2	5.2		5.2	
Eco R1/BAM H-1	5.7		5.7		
	5.2			5.2	
			4.7		4.7
Eco R1/Xbal			6.4		6.4
	5.1		5.1	5.1	

a The numbers represent the sizes of restriction fragments in kb from Southern blots of the respective DNAs hybridized with a cloned C_K probe (see Fig. 1).

b See Refs. 8 and 9.

c NS-1n is a subline of NS-1 which has lost the 5.2kb Bam kappa gene (8).

d G has only one Bam-H1 site flanking C_K within the Eco R1 sites which delineate the mouse DNA insert, the other Bam-H1 site is within the short arm of Ch.4A (see Fig. 7).

genes (9). Both Eco R1/Bam H1 and Eco R1/Xba1 digests of clone G DNA give rise to C_κ restriction bands which have the same size as the "new" NS-1n bands. Thus, although clone G was derived from MOPC-21, it is like a gene of the myelomas NS-1 and NS-1n. The latter were derived by subcloning of a MOPC-21 cell line (29); possibly a minor cell population present in MOPC-21 was selected. If this is true this cell population may still exist in the MOPC-21 myeloma carried in our laboratory and may have given rise to clone G. Alternatively clone G may have been derived from either of the two major MOPC-21 genes during cloning (see Discussion).

The Sp101 clone has a C_κ restriction map which is identical (Fig. 7) to that which we previously determined for liver (6) and Sakano et al. for embryonic DNA (2) and is therefore a clone of a germline C_κ gene.

The three clones represent three forms of the C_κ gene: a germline C_κ gene (Sp101), a rearranged "functional" C_κ gene (B1), and a "nonfunctional" rearranged C_κ gene which apparently does not code for kappa chains (G) (see Discussion). They are therefore suitable for the comparison of the fine structure of a "functional" with "nonfunctional" kappa genes.

Electron microscopy of cloned DNAs. Restriction enzyme analysis indicated that all three clones were oriented so that the Eco R1 site 5' of the C_κ gene was linked to the short arm of Ch.4A. Heteroduplexes between the cloned DNAs Sp101 and B1 showed that the molecules are homologous except for a short portion adjacent to the short arm of Ch.4A (Fig. 2a). Restriction enzyme analysis demonstrated that the B1 insert was slightly longer than the Sp101 insert (Fig. 7); the two single stranded arms of this heteroduplex can therefore be assigned as 1537 ± 78 and 1961 ± 98 bp for Sp101 and B1 respectively (Table II). When either Sp101 or B1 were heteroduplexed with G, two single stranded arms were again seen adjacent to the short arms of Ch.4A (Fig. 2b and d). In addition a single stranded deletion bubble was observed 3' of the single stranded arms (Figs. 2b and d). Rare open molecules (Figs. 2c and e), showed that the bubble was due to a deletion of approximately 970 bp in clone G (Table II): the larger single stranded arm of the open molecules in Sp101 x G heteroduplexes has approximately the same length as the sum of the lengths of the single stranded Sp101 arm, the homology region (between the single stranded arms and the deletion bubble), and the bubble. Conversely the short arm of the open molecules has only the length of the sum of the single stranded arm of G and the length of the homology region (compare columns 5 and 6 of Table

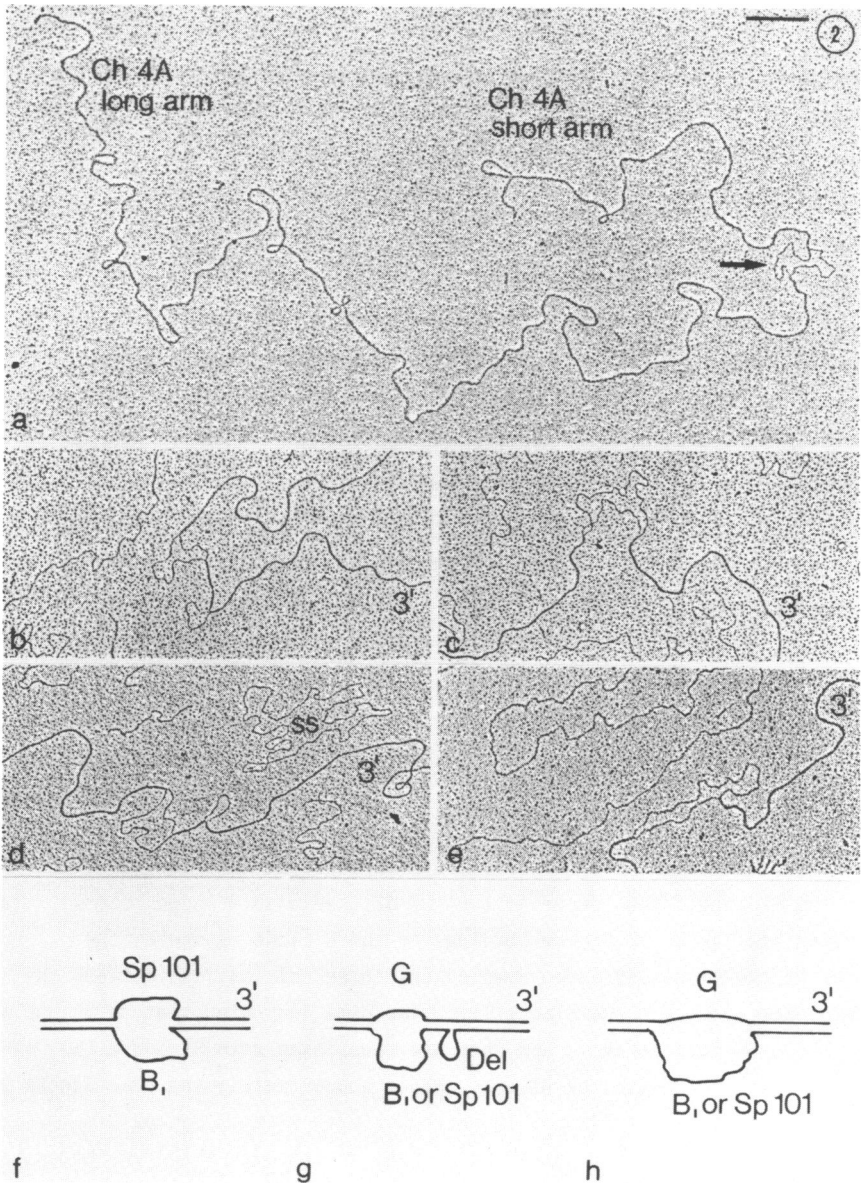


Figure 2. Heteroduplex analysis of cloned DNAs. a. Sp101 and B1: → single stranded nonmatching sequences. b. and c. Sp101 and G: b. shows an example where the homology region between the deletion bubble and the single stranded arms is hybridized, c. shows a rare open molecule. d. and e.: the two different types of heteroduplexes formed between B1 and G. f. to h.: Schematic drawings of the three types of heteroduplexes observed. Bar=1kb. ss = single stranded marker DNA.

Table II. Heteroduplex Analysis

1 Combination of cloned DNAs	2 Single Stranded arms[a]	3 Homology region[b]	4 Deletion bubble	5 Sum of 2-4	6 Single stranded arms in open molecules
Sp101					
with B1	1537±78				
with G	1590±95	635±75	968±88	3193	2966
B1					
with Sp101	1961±98				
with G	1936±176	521±113	978±62	3435	3587
G					
with Sp101	694±53	635±75	968±88	2297	1253
with B1	799±143	521±113	978±62	2298	1413

a The numbers represent the number of bases (or base pairs) ± standard deviation of the particular cloned DNA underlined in Column 1 when it is heteroduplexed with the indicated DNA. Measurements were made on 13, 22 and 23 heteroduplexes of G x B1, B1 x Sp101, and G x Sp101, respectively.

b Homology region = ds DNA between the ss arms and the deletion bubble.

II). The same principle applies to the B1 x G heteroduplexes (Table II).

Clone B1 represents the expressed gene of MOPC-21 which codes for the kappa chains produced by this myeloma (see above). Since the MOPC-21 kappa chain contains the J2 sequence (23,2,3) one would expect B1 to have joined the V_{21} gene to the 5' side of J2. The divergence between the germline clone and B1 should thus occur just 5' of J2 (see also R loops, below). To determine how far 5' of C_{κ} the homology of the G clone extended, the lengths of the single stranded arms of the three combinations between the three clones were compared (Table II). The data indicated that Sp101 has a 1537 ± 78 bp single stranded arm when heteroduplexed with B1 and a 1590 ± 95 bp arm with G. Likewise the B1 arms showed approximately the same length 1961 ± 98 versus 1936 ± 176 bp, in heteroduplexes with Sp101 and G respectively. The 5' portion of the G clone insert does therefore begin to diverge from the germline sequence also at or around J2. Analysis by R looping (see below) confirmed that G contains J2.

The homology region of the three clones between J2 and where the deletion begins in G is approximately 521 to 635 bp (Table II). This puts the beginning of the deletion of G in the vicinity of the J4 gene (see Figure 7 and Discussion).

The short arm of Ch.4A contains 10 886 bp (10). We measured 10,543 ± 463, 10,724 ± 529, and 10,857 ± 514 bp between the open arms and the short end of the cloned DNA in the three heteroduplex combinations respectively. This suggested that the three inserts had no substantial homology 5' of J2, which was directly confirmed by heteroduplexing of the inserts isolated after Eco R1 cleavage of the cloned DNAs. In all three combinations only open ended molecules, such as illustrated in Fig. 3, were seen.

According to the heteroduplex results (see above) the deletion in clone G should have eliminated J5. In order to assess this directly, R loops were formed with MOPC-167 kappa mRNA which contains J5 (30-32). Both Sp101 and B1formed C_κ R loops with MOPC-167 mRNA and double stranded DNA loops which correspond to the IVS between C_κ and J5 (Figs. 4a, b, and 1). The average lengths of the DNA loops were 2390 and 2340 bp in Sp101 and B1, respectively. This agrees with the C_κ to J5 distances of 2.35 to 2.5kb determined by others (2-4). R loops between clone G DNA and MOPC-167 kappa mRNA showed only a C_κ R loop (Fig. 4c and j). No DNA loop was found among 77 observed R loops. At least half of these had an RNA molecule which was undegraded, i.e., more than long enough to contain the J5 segment. This strongly indicates the absence of J5 in clone G.

In order to further assess whether clones B1 and G contained the J2 sequence, R loops with PC-3741 and MOPC-21 kappa mRNAs were formed. Both of these RNAs contain J2 (2,3,23,33). With MOPC-21 mRNA (Fig. 4d and 1) and PC-3741 mRNA (not shown) the germline clone Sp101 formed a C_κ R loop and a ds DNA loop (IVS + J2-J5) of an average length of 3.2kb with PC-3741 and

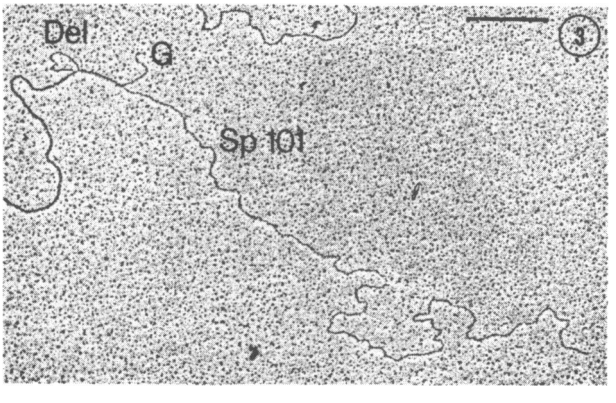
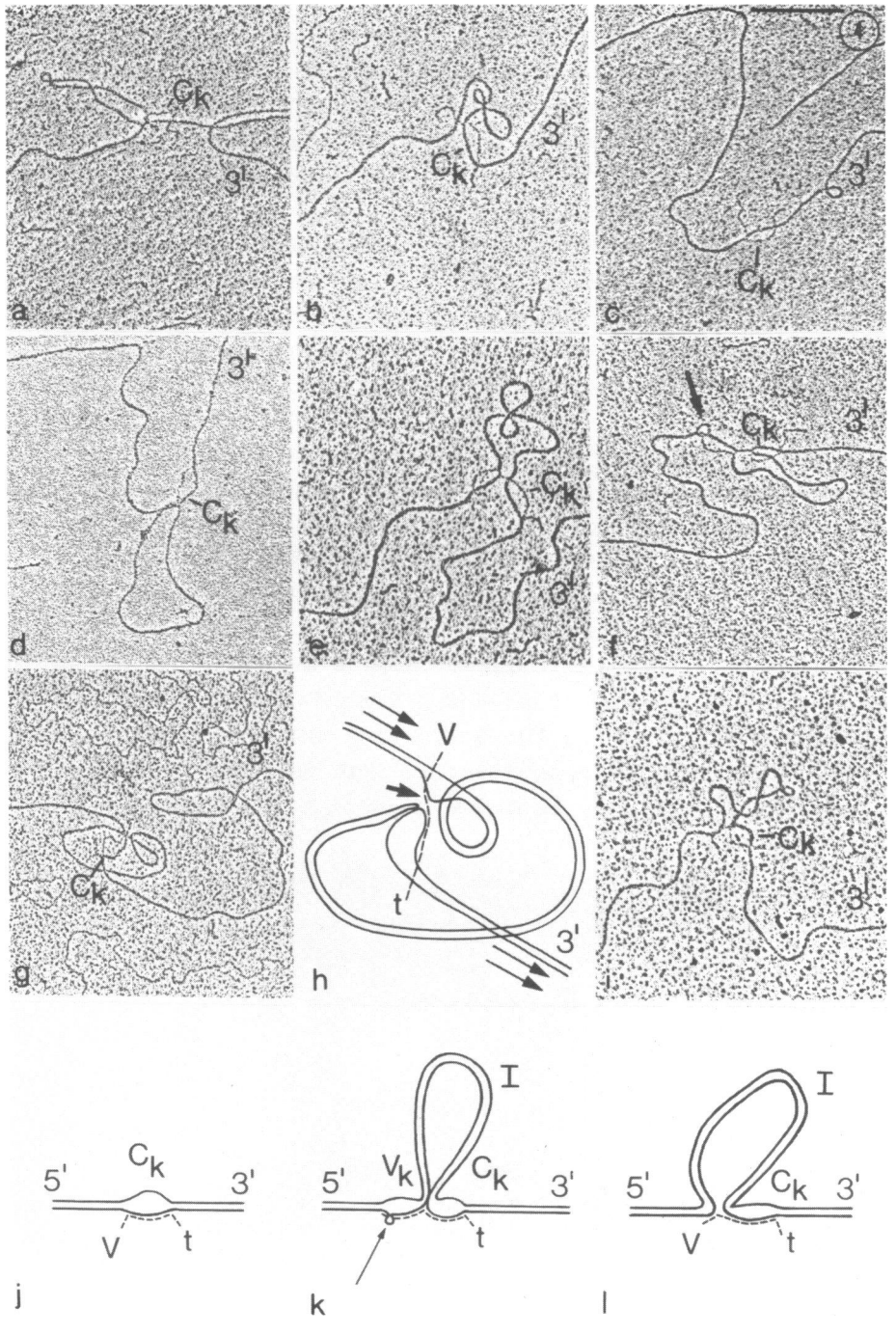


Figure 3. Heteroduplex analysis of full length DNA of clone Sp101 and the Eco R1 excised kappa gene insert of clone G. Bar = 1kb.


a
Ck
3'
b
Ck
3'
c
3'
Ck
d
3'
Ck
e
Ck
3'
f
Ck
3'
g
3'
Ck
h
V
t
3'
i
Ck
3'
j
5'
Ck
3'
V
t
k
I
5'
Vk
Ck
3'
t
l
I
5'
Ck
3'
V
t

3.4kb with MOPC-21 RNA indicating that, as expected, it contained J2. The length measurements of R loops vary more than those of heteroduplexes because of branch migration. Nevertheless the distance J2-C_κ agrees with the 3.2 to 3.5kb length found by others (2-4).

The PC-3741 and MOPC-21 V regions belong to different V kappa subgroups (34) and are thus sufficiently mismatched that their respective nucleotide sequences should not cross react. R loops of PC-3741 RNA with B1 DNA will, therefore, measure the presence of J2 alone. With PC 3741 κmRNA both B1 and G formed a C_κ R loop; they also formed a ds DNA loop of 3.2 and 2.3kb, respectively (Fig. 4g, h, i, and l). This shows that both contain J2. The foreshortened ds DNA loop of G further confirms the deletion.

With 14S mRNA (size of κmRNA) prepared from the total cytoplasmic poly A RNA of MOPC-21 by size purification using sucrose gradients and formamide gels, clone B1 DNA showed the expected R loops with the C and V regions (Fig. 4e and k). The ds DNA loop between C_κ and J2 was 3.38 ± 0.145kb.

When clone G DNA was R looped with this MOPC-21 kappa RNA preparation it was surprising to also find two R loops, a C_κ and a V kappa loop with an intervening double stranded DNA loop (Fig. 4f and k). Since the DNA of clone G did not hybridize on Southern blots with a MOPC-21 V region cDNA (Fig. 6) and since this region of clone G did not anneal with clone B1 DNA in heteroduplexes, it was not expected to contain the V_{21} sequence. Two possibilities were considered, either clone G contains a V gene similar to V_{21}, or MOPC-21 cytoplasmic RNA contains two κ RNAs with different V regions. The former appeared less likely when also under nonstringent conditions the B1 and G DNAs did not anneal in the area of their V regions (data not shown). Clone G has a complete V region gene since a small single stranded bubble corresponding to the IVS within the signal peptide sequence (4) was seen in many R loops (Fig. 4f and k, arrow). We have found that clone G contains a distinct V region, apparently unrelated to that of the kappa chains of MOPC-21 (Walfield and Storb, in preparation; see Discussion). The double stranded DNA loop formed by the IVS 5' of C_κ and

Figure 4. R loop analysis of cloned DNAs. Hybridization with MOPC-167 kappa mRNA (contains J5) and DNA of clones Sp101 (a), B1 (b) and G (c). Hybridization with MOPC-21 total cytoplasmic kappa RNA and DNA of clones Sp101 (d), B1 (e), and G (f). Hybridization with PC-3741 kappa mRNA (contains J2) and DNA of clone B1 (g; h is a schematic drawing of g) and clone G (i). Schematic drawings of the three different types of kappa R loops: a single C_κ R loop (j) as shown in c; two R loops and an IVS(I) DNA loop (k) as shown in e and f; a C_κ R loop and and IVS DNA loop (l) as shown in a, b, d, g, and i. Bar = 1kb, t = poly(A) tail, V=V region of κmRNA.

the intervening J sequences in G DNA x MOPC-21 RNA hybrids was on the average 2.67 ± 0.239 kb long, i.e., considerably shorter than that with Sp101 or B1 DNA, again confirming the deletion in G.

Finally, the possibility of an aberrant J1 sequence in the DNA of clone G was investigated. R loops were formed with MOPC-41 kappa mRNA which contains J1 (3,4,35). Among 60 observed R loops between G DNA and MOPC-41 RNA (appearance like Fig. 4c), only C_K R loops were found while 25% of the C_K R loops of Sp101 DNA had an attached double stranded DNA loop (not shown; the structures look like the scheme Fig. 4 1). Thus, while the germline clone Sp101 possesses J1 as expected, G does not contain J1. This further supports the observation described above, that the V sequence in G is joined close to J2.

We obtained a direct illustration of the presence of a V region on the portions of the B1 and G DNAs which are single stranded in heteroduplexes (Fig. 5). MOPC-21 total cytoplasmic kappa mRNA was added under R loop conditions to heteroduplexes between G and Sp101 DNAs or G and B1 DNAs. In the former situation many of the heteroduplexes showed a double stranded G arm where the RNA had hybridized, and within it a small single stranded DNA bubble indicative of the signal peptide IVS (Fig. 5a and b). A C_K R loop was present in the majority of the heteroduplexes. Some complicated structures were also seen, where one RNA molecule combined with both C_K and the single stranded DNA arm of G (not shown). Probably due to strand migration in the hybrid shown in Fig. 5a the 3' end of the G arm is single stranded. In the majority of such molecules, however, the 3' end of the hybridized G arm was completely double stranded (Fig. 5d) suggesting that the V region may be close to or directly associated with J2. Fifteen hybrids of the MOPC-21 RNA with the G arm were seen, while no hybrids, as expected, were observed with the Sp101 arm.

When MOPC-21 total cytoplasmic kappa mRNA was added to B1 x G heteroduplexes, molecules were found which had the RNA associated with either the B1 (Fig. 5c, e and f) or the G arm (Fig. 5g). Except in cases of probable branch migration and/or partial RNA degradation, the RNA appeared flush with either the 3' end of the G arm (Fig. 5g) or the B1 arm (Fig. 5e). Thus the V regions begin at or very close to where B1 and G diverge. Small bubbles of the signal peptide IVS were seen on hybrids of either arm (Fig. 5e, f, and g).

DISCUSSION

The combined information obtained from restriction mapping and

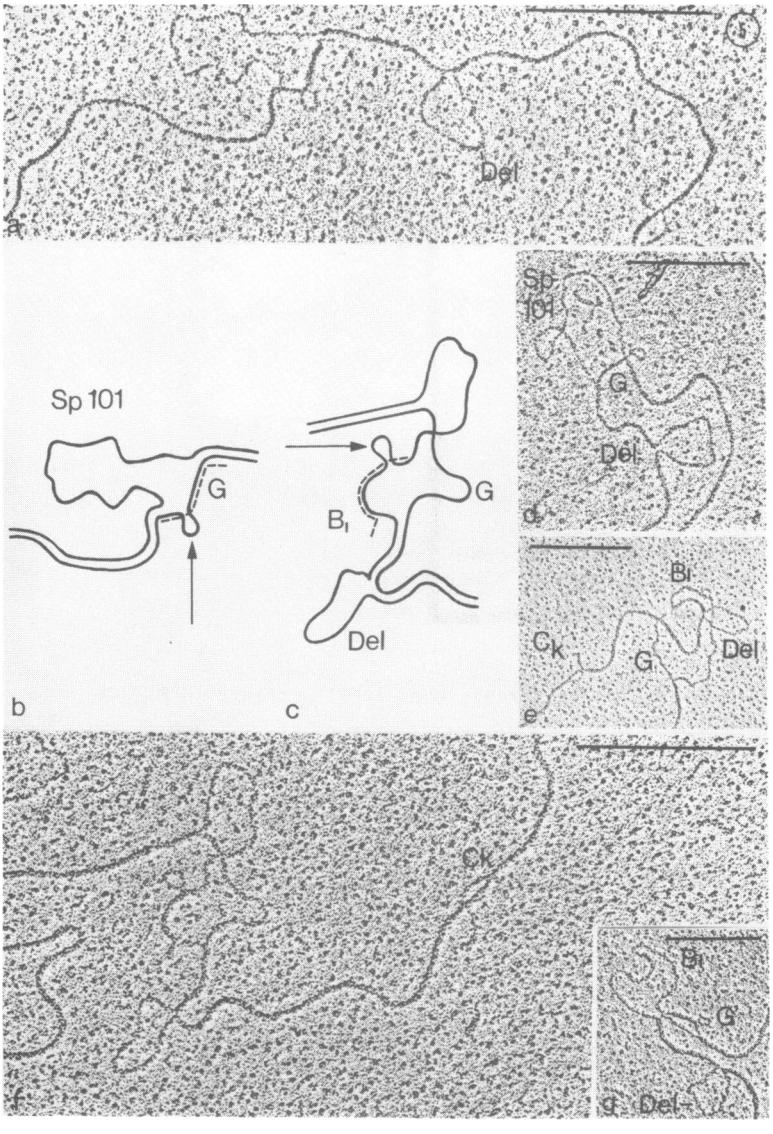


Figure 5. Combination of heteroduplexing and hybridization with MOPC-21 kappa RNA. Heteroduplexes were first formed, then RNA was added and R loop conditions were established. DNA of clones Sp101 and G (a, b, and d); DNA of clones B1 and G in one orientation with a V region of the RNA hybridized to the single stranded B1 arm (e, f and c), or in the other orientation with a V region of the mRNA hybridized to the G arm (g). Del.=deletion bubble. C_K=R loop with the C gene. The arrows in b and c point at the single stranded DNA loop formed by the IVS within the signal peptide gene sequence. Bars=1kb.

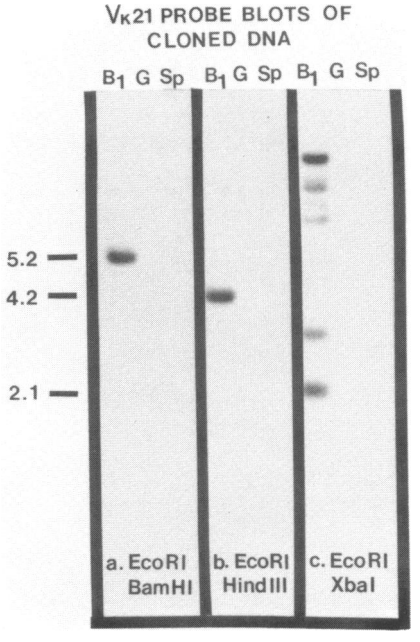


Figure 6. Autoradiogram of Southern blots of cloned DNAs with a V_{21} probe. The fragments of B_1 DNA digested with Eco R1-Xbal which are larger than 2.1 kb are due to incomplete digestion.

electronmicroscopy of the three clones is illustrated in Fig. 7. The restriction map of the germline clone, Sp101, agrees with the maps of Sakano et al. (2) and Max et al. (3). Clone B1 corresponds to the functional gene coding for the MOPC-21 kappa chains. It has the V_{21} gene joined at J2 in a functional V-J joining. Clone G corresponds to the 5.9kb Bam kappa gene of the myelomas NS-1 and NS-1n. It has a V_G gene also joined around J2. Sequencing studies in progress will show whether or not this joining is in a proper translational reading frame. The V_G sequence is apparently unrelated to V_{21}, since DNAs of clones B1 and G do not anneal in this region. Furthermore, a fragment corresponding to 300 bp 3' of the Eco R1 site at the 5' end of the clone G insert (Fig. 7E-1) does not hybridize in Southern blots with B1 nor with the 5.2kb Bam gene of MOPC-21 (Walfield and Storb, in preparation). It does hybridize, however, with the 6.9kb Bam gene of MOPC-21, the 6.9 and 5.9kb Bam genes of NS-1n; and to a V_G subgroup of at least six germline genes which is quite distinct from the V_{21} subgroup (Walfield and Storb, in preparation). It thus appears that the 6.9 Bam genes of MOPC-21 and NS-1 are related to the 5.9 Bam gene of NS-1. Our results agree with the possibility that the 5.9 gene was derived by deletion of about 1kb from the 6.9 Bam gene. When MOPC-21 DNA is analyzed in

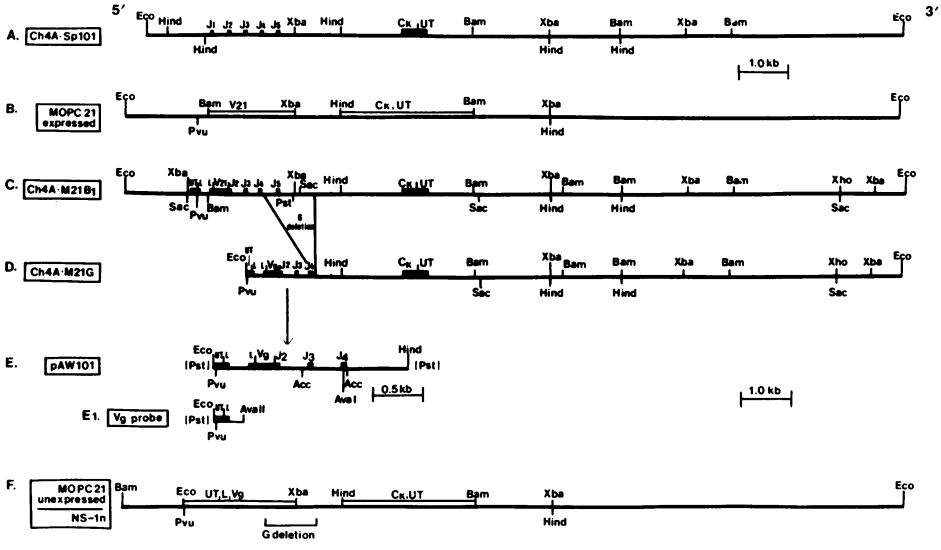


Figure 7. (A) Restriction enzyme, heteroduplex and R loop map of the insert in clone Charon 4A-Sp101. The bars represent R loops formed with κ mRNAs to J regions 1-5 (the positions of J3 and J4 were assigned by alignment with the published sequence (2) and with the C_K region and the 3' untranslated region (UT). (B) Restriction enzyme map of the MOPC-21 gene coding for kappa chains, derived from Southern blots of MOPC-21 myeloma DNA (Fig. 1 and Ref. 6). Fragments which contain the variable and C_K sequences of MOPC-21 are shown with double lines. (C) Map of the insert in clone Charon 4A-M21 B1. Shown here are the regions for the 5' untranslated region (UT), the signal peptide (leader) sequence (L), the variable sequence of MOPC-21 (V_{21}), the J regions, and the C_K and 3' UT sequences. (D) Map of the insert in Charon 4A-M21 G. 5' UT, L, and the variable G region (Vg) sequences are homologous to MOPC-21 "G" mRNA. (E) Map of pAW101, the 5' Eco R1/Hind III fragment of Ch4A-M21G subcloned into pBR322. The Eco R1 and Hind III restriction sites have been altered to PstI sites. (E1) Shows the 300 base pair fragment cut from the 5' end of pAW 101. (F) Restriction enzyme map of the unexpressed MOPC-21 gene, and the kappa genes found in NS-1n. The NS-1n gene is present both with and without a "G-like" deletion. Restriction endonucleases used were Acc I, Ava I, Ava II, Bam HI, Eco R1, Hind III, Pst I, Pvu II, Sac I, Xba I, and Xho I.

Southern blots, it contains no trace of the 5.9 Bam gene (Fig. 1), nevertheless, clone G with the deletion was derived from this DNA. Either the deletion had occurred in the myeloma in a small subset of cells, whose DNA happened to give rise to clone G, or the 6.9 Bam gene was actually cloned and the deletion occurred during propagation of the phage. The latter possibility is likely since no recombinant phage with the 6.9 Bam gene was obtained from the MOPC 21 library which suggests that the deleted sequence is

unstable in the Charon 4A cloning system. The deletion genes in clone G and myeloma NS-1 (the 5.9 Bam gene) appear to have the same size and restriction maps in Southern blots. This does not prove that the deletion comprises exactly the same nucleotides of the genes - a possible microheterogeneity is now being assessed by DNA sequencing.

Heteroduplex analysis indicates that the beginning of the deletion in G is approximately 600 bp 3' from the beginning of J2. This puts it just 5' of J4 (2, 3). However, by restriction mapping, G exhibits AccI and Ava I sites like those inside and just 3' of J4 respectively (Fig. 7). Due to the limits of accuracy of heteroduplex measurements we therefore assume that the deletion in G begins just 3' of J4 and extends for about 1kb 3'. This deletes Xba I, Pst I, and Sac I sites within the large intron. Thus the C_κ containing Xba I fragment present in germline and all "normal rearranged" C_κ genes is replaced by a larger Xba I fragment (Fig. 1, Table I and Fig. 7).

We do not know yet whether the deletion gene in NS-1 and NS-1n is transcribed and the transcript processed. There are transcripts of the size of mature kappa mRNA containing the V_G sequence in both MOPC-21 and NS-1n (Figs. 4 and 5 and Walfield and Storb, in preparation). It is most likely that these are derived from the 6.9 Bam gene in MOPC-21 and are probably also coded for by the 6.9 Bam gene in NS-1n. The activity of the 5.9 Bam gene is currently being assessed in our laboratory. The deletion eliminates a possible internal splice site, AGGT (36,37) present in normal kappa genes just 5' of the Xba I site (2). It is thus possible that the 5.9 Bam gene may be transcribed, but the RNA not processed.

The 6.9 and 5.9 Bam genes do not code for κ chains (8). By definition they are thus nonfunctional rearranged genes. We assume because of the relationships discussed above, that the 6.9 Bam gene is the same as clone G, except for the deletion in the latter. Since the 6.9 gene codes for mature sized κRNA it can be transcribed and the transcript can be processed in some way. The G-κ RNA probably contains the sequence for a signal peptide since the intron within this sequence is present in the gene (Fig. 5a). Thus if a κ protein were translated from this RNA it should find it's way into the rough endoplasmic reticulum. Preliminary results suggest that the G-κ RNA is present in relatively large quantity in the whole cytoplasm but sparse or absent in membrane bound ribosomes of MOPC-21 and NS-1n (Storb, unpublished). It remains to be determined whether the RNA is capable of cell free translation. It may lack a ribosome binding site in functional

relationship to an initiation codon due to a defect in the gene or in the RNA processing, or it may have suffered from a faulty V-J joining which could cause alterations in reading frame.

The 6.9 Bam gene seems to be an example of a "nonfunctional" rearrangement which manifests it's inactivity only at one of the last steps in the processing sequence from gene to protein. It appears that "allelic exclusion" of immunoglobulin genes may be generally guaranteed not because a second rearrangement cannot occur in a lymphoid cell, but because of the complexity of the processes of gene expression which make two functional rearrangements, and therefore a scramble of antibody molecules, unlikely.

ACKNOWLEDGMENTS

U.S. is very grateful to W.W. Franke for generously providing advice, space and equipment to carry out part of this project as well as to W. Roevekamp and W. Schmid and the German Cancer Research Center. U.S. is also indebted to H. Swift for first instructions in R looping and to K. Mähler for photography. We thank F. Blattner for providing the cloning strains and the cloning procedure prior to publication, M. Potter, C. Milstein and M. Weigert for mouse tumors, P. Early and L. Hood for p167kR1, R. Wilson and B. Arp for MOPC-21 and NS-1n DNAs, F. Blattner and S. Tilghman for advice and A. Raphael for typing the manuscript. Some early experiments were done in the P3 facility of the University of California, San Francisco, for which we are grateful to H. Goodman and the Dept. of Biochemistry and Biophysics, UCSF. A.M.W. was a recipient of a NSF postdoctoral fellowship. The project was supported by grants CA/AI-25754 and DE-02600 from NIH, and grant PCM 78-13205 from NSF, as well as by the German Cancer Research Center, Heidelberg.

**To whom correspondence should be sent.

REFERENCES

1. Abbreviations used: V(C) genes - genes coding for the variable (constant) region of immunoglobulins. bp - base pairs. kb - kilobases. ds and ss - double and single stranded. IVS - intervening sequence. J - joining gene (Refs. 2,3). V_{21} - V gene coding for the MOPC-21 kappa chain. V_G - V gene of clone G DNA.
2. Sakano, H., Hüppi, K., Heinrich, G. and Tonegawa, S. (1979) Nature 280, 288-294.
3. Max, E.E., Seidman, J.G. and Leder, P. (1979) Proc. Natl. Acad. Sci. USA 76,3450-3454.
4. Seidman, J.G., Max, E.E., and Leder, P. (1979) Nature 280, 370-375.

5. Lenhard-Schuller, R., Hohn, B., Brack, C., Hirama, M. and Tonegawa, S. (1978) Proc. Natl. Acad. Sci. USA 75, 4709-4713.
6. Wilson, R., Miller, J. and Storb, U. (1979) Biochemistry 18, 5013-5012.
7. Steinmetz, M. and Zachau, H.G. (1980) Nucl. Acids Res. 8, 1693-1706.
8. Storb, U., Arp, B., and Wilson, R. (1980) Nucl. Acids Res., in press.
9. Wilson, R., Storb, U., and Arp, B. (1980) J. Immunol. 124, 2071-2076.
10. Blattner, F.R., Williams, B.G., Blachl, A.E., Denniston-Thompson, K., Faber, H.E., Furlong, L., Grunwald, D.J., Kiefer, D.O., Moore, D.D., Schumm, J.W., Sheldon, E.L. and Smithies, O. (1977) Science 196, 161-169.
11. Maniatis, T., Hardison, R.C., Lacy, E., Lauer J., O'Connell, C., Quon, D., Sim, G.K. and Efstratiadis, A. (1978) Cell 15:687-701.
12. Blattner, F.R., Blechl, A.E., Denniston-Thompson, K., Faber, H.E., Richards, J.E., Slightom, J.L., Tucker, P.W. and Smithies, O. (1978) Science, 202, 1279-1284.
13. Benton, W.D. and Davis, R.W. (1977) Science 196, 180-182.
14. Joho, R., Weissman, I.L., Early, P., Cole, J., and Hood, L. (1980) Proc. Natl. Acad. Sci USA 77, 1106-1110.
15. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
16. Wickens, M.P., Buell, G.N. and Schimke, R.T. (1978) J. Biol. Chem. 253, 2483-2495.
17. Bolivar, F., Rodriguez, R.L., Greene, P.J., Betlach, M.C., Heyneker, H.L., Boyer, H.W., Crosa, J.H. and Falkow, S. (1977) Gene 2, 95-113.
18. Selsing, E. and Wells, R.W. (1979) Nucl. Acids Res. 6, 3025-3040.
19. Curtiss, R. III, Pereira, D.A., Hsu, J.C., Hull, S.C., Clark, J.E., Maturin, L.J., Sr. Goldschmidt, R., Moody, R., Inoue, M. and Alexander, L. (1977) Recombinant Molecules: Impact on Science and Society, Beers, R.F., Jr. and Bassett, E.G., Eds., pp. 45-56 Raven Press, New York.
20. Grunstein, M. and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3961-3965.
21. Barnes, W.M. (1977) Science 195, 393-395.
22. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74-5463-5467.
23. Svasti, J. and Milstein, C. (1972) Biochem. J. 128, 427-444.
24. Storb, U. (1978) Proc. Natl. Acad. Sci. 75, 2905-2908.
25. Near, R. and Storb, U. (1979) Biochem. 18, 964-972.
26. Davis, R., Simon, M., and Davidson, N. (1971) In Methods in Enzymology, 21D, L. Grossmann and K. Moldave, Eds. pp. 413-428 Academic Press, New York.
27. Thomas, M., Wite, R.L., and Davis, R.W. (1976) Proc. Natl. Acad. Sci. 73, 2294-2298.
28. Herrmann, R., Neugebauer, K., Zentgraff, H., and Schaller, H. (1978) Molec. Gen. Genet. 159, 171-178.
29. Köhler, G., Howe, S.C. and Milstein, C. (1976) Eur. J. Immunol. 6, 292-295.
30. Hood, L., McKean, D., Farnsworth, V., and Potter, M. (1973) Biochemistry 12, 741-749.
31. Rudikoff, S., and Potter, M. (1978) Biochemistry 17, 2703-2707.
32. Kabat, E.A., Wn, T.T., and Bilofsky, H. (1979) In Seuqences of Immunoglobulin Chains, U.S. Dept. of Health, Education and Welfare, NIH Publ. No. 80-2008.
33. Weigert, M., Gatmaitan, L., Loh, E., Schilling, J., and Hood, L. (1978) Nature 276, 785-790.
34. Potter, M. (1977) Adv. Immunol. 25, 141-207.
35. Gray, W.R., Dreyer, W.J., and Hood, L. (1967) Science 155, 465-467.

36. Benoist, C., O'Hare, K., Breathnach, R. and Chambon, P. (1979) Nucl. Acids Res. 8, 127-141.
37. Seif, I., Khoury, G., and Dhar, R. (1979) Nucl. Acids Res. 6, 3387-3398.