MOLECULAR AND CELLULAR BIOLOGY, July 1985, p. 1660–1675
0270-7306/85/071660-16$02.00/0

Vol. 5, No. 7

Nonproductive Kappa Immunoglobulin Genes: Recombinational Abnormalities and Other Lesions Affecting Transcription, RNA Processing, Turnover, and Translation

D. E. KELLEY,[1]* L. M. WIEDEMANN,[1] A.-C. PITTET,[1] S. STRAUSS,[1] K. J. NELSON,[1] J. DAVIS,[2] B. VAN NESS,[2] AND R. P. PERRY[1]*

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111,[1] and Biochemistry Department, University of Iowa, Iowa City, Iowa 52240[2]

Received 12 February 1985/Accepted 9 April 1985

Six nonproductive kappa immunoglobulin genes (κ^- alleles) were cloned and sequenced. The structural abnormalities discerned from sequence analysis were correlated with functional lesions at the level of transcription, RNA processing, turnover, and translation. Four κ^- alleles, three containing V_κ genes and one not, are transcribed at normal or even greater than normal rates, the defects in these genes being expressed at various posttranscriptional levels. The other two κ^- alleles, both of which lacked V genes, exhibited greatly depressed yet clearly detectable transcriptional activity. These results are consistent with a hierarchical relationship between enhancer and promoter elements in which the enhancer establishes transcriptional competence at the κ locus and the promoter (or pseudopromoter) determines the relative level of transcriptional activity. One of the structural abnormalities discovered in this study, a large deletion which removes the entire J_κ region, also provides new insight into the mechanism of VJ and VDJ recombination.

The genetic elements specifying the variable-region (V) and constant-region (C) portions of immunoglobulins are well separated in germline DNA and specifically rearranged during B lymphocyte maturation to create functional immunoglobulin genes (53). However, many of the rearranged genes are incapable of producing useful immunoglobulins either because of an aberration in the recombination process or a deleterious somatic mutation or because they involve an intrinsically defective recombining element, e.g., a pseudo V gene (3, 4, 7, 15, 21, 22, 26, 48, 58). In plasma cell tumors, chromosomal translocations also contribute to the pool of nonproductive immunoglobulin alleles (38). About a third of the rearranged κ alleles in mouse plasmacytomas (PCs) are judged nonproductive, the causes of nonproductivity being found at virtually every level of gene expression from deficient transcription to the formation of faulty protein (3, 7, 10, 21, 26, 40, 41, 48, 58).

The nonproductive (κ^-) alleles constitute a potential source of natural mutants which can be used to define the features of immunoglobulin gene structure that are critical for expression. For example, they may be used to examine the relationship between promoter and enhancer functions in transcriptional regulation (39). Although such mutants may lack the precisely specified modifications of synthetically engineered genes that are reintroduced into cells via transfection, they have a distinct advantage over the latter in that they are at their normal chromosomal location and should therefore be subject only to the particular influences and constraints associated with the immunoglobulin locus.

In the present study we have examined the sequence organization and RNA production of the κ^- alleles in six selected mouse PCs. These particular κ^- genes were provisionally classified as transcriptionally defective because we could not attribute any specific κ transcripts to them in previous analyses of the PC nuclear RNAs (41). However, as our present detailed studies will demonstrate, only two of these κ^- genes have markedly depressed transcriptional activity; the others are actually transcribed at normal or even greater than normal rates, but are nonproductive for a variety of other reasons. By elucidating the structural lesions in the κ^- genes and determining their consequences for transcriptional efficiency, RNA processing, and transcript stability, we have gained new insight into the functional attributes of various parts of the κ gene structure. In addition, these studies have revealed a novel deletion-generating rearrangement which has an important implication for mechanistic models of immunoglobulin gene recombination.

MATERIALS AND METHODS

DNA and polyadenylated [poly(A)$^+$] nuclear RNA were prepared from a series of NZB mouse PCs (41) and used for Southern blot, Northern blot, and S1 nuclease protection analyses as previously described (35, 57). DNA was sequenced by the Maxam and Gilbert procedure (33).

For cloning the κ^- alleles, PC DNA was digested to completion with an appropriate restriction enzyme (New England BioLabs, Inc.) and electrophoresed on a 0.56% low-melting-point agarose gel (Seakem). Fragments of selected sizes were extracted from the gel, concentrated by ethanol precipitation, and ligated to a suitable pair of phage arms (Table 1) with bacteriophage T4 DNA ligase. The ligation products were packaged into phage particles (20) and grown in *Escherichia coli* strain HB101(λ1059Ch30), BHB2600, or LE392. Lysates were successively screened (5) to single-plaque purity with the probes listed in Table 1. Selected fragments of insert DNA were isolated, ligated to plasmid vectors (pBR322 and the pUC series), and used to transfect a competent strain of *E. coli*.

The nuclei used for transcription measurements were isolated by the method of Schibler et al. (47) and stored in 50% glycerol buffer at −70°C. Such nuclei exhibited uniform levels of activity for at least 4 months after isolation. Elongation reactions (47) were carried out for 10 min at 26°C.

* Corresponding author.

TABLE 1. Status of κ alleles in a series of mouse PCs and characteristics of the cloned κ^- alleles

Genotype and PC	κ^+ allele		κ^- allele				
	V_κ	J_κ[a]	Germline C_κ^+ *Bgl* and *Xba* fragments	Fragment cloned[b] (kb)	Phage vector	Screen probe(s)[c] +	Screen probe(s)[c] −
κ^+/κ^-							
2154	21G	4	+	7.5 *Bam*HI	λCh28	c	
3386	21A	2	−	8.5 *Bam*HI	λCh30	c and e	V_κ21
6684	21E	1	+	4 *Hin*dIII	λ1059Ch30	d	V_κ21
7183	21D	4	−	10 *Hin*dIII	λ1059Ch30	c	
8701	21D	3	−	9.5 *Bam*HI	λCh28	c	
10916	21C	3	−	7 *Sac*I	λgt WES·λB	c	V_κ21
κ^+/κ^0							
3741	21C	2					
6308	21D	1					
7043	21D	3					
7461	21G	1					

[a] In conformity with previous publications from this laboratory, we have enumerated only the four functional J_κ elements. J_κ3 and J_κ4 are often termed J_κ4 and J_κ5 by other authors.
[b] The cloned κ^- fragments are listed by size and the restriction enzyme used to obtain the fragment. The sources of the λ phage vectors were F. Blattner for Ch28 and Ch30 (45), P. Leder for λgtWES·λB (27), and K. Marcu for the composite of λ1059 (24) and Ch30.
[c] The κ^0 probes used for the positive (+) screen are shown in Fig. 1.

RNA was extracted and hybridized with nitrocellulose strips containing immobilized DNA as described previously (30), except that dextran sulfate was omitted from the hybridization solution. The labeled RNA is apparently nicked during manipulation of the nuclei so that its length, as judged by electrophoretic analysis on glyoxal gels (data not shown), averages about 300 to 600 nucleotides. Hybridizations were routinely done with duplicate strips incubated with a twofold difference in RNA input. In all cases the amount of RNA hybridized was proportional to the RNA input, indicating that the hybridizing DNA sequences were in satisfactory excess. This was confirmed in one set of experiments in which a duplicate batch of nuclei was treated with RNase before the elongation reaction to remove potentially competitive RNA (47). The results obtained with these nuclei were similar to those obtained with untreated nuclei, providing additional verification that the DNA excess was adequate to render the measurements insensitive to possible differences in the specific activity of the hybridizing RNA.

RESULTS

Cloning and sequencing of the κ^- genes. The six PCs selected for these studies all express κ chains bearing variable regions of the V_κ21 group. By using the results of previous studies (40, 55), each of these PCs could be characterized with respect to the particular V_κ21 gene and J_κ element used in the formation of the productive (κ^+) allele

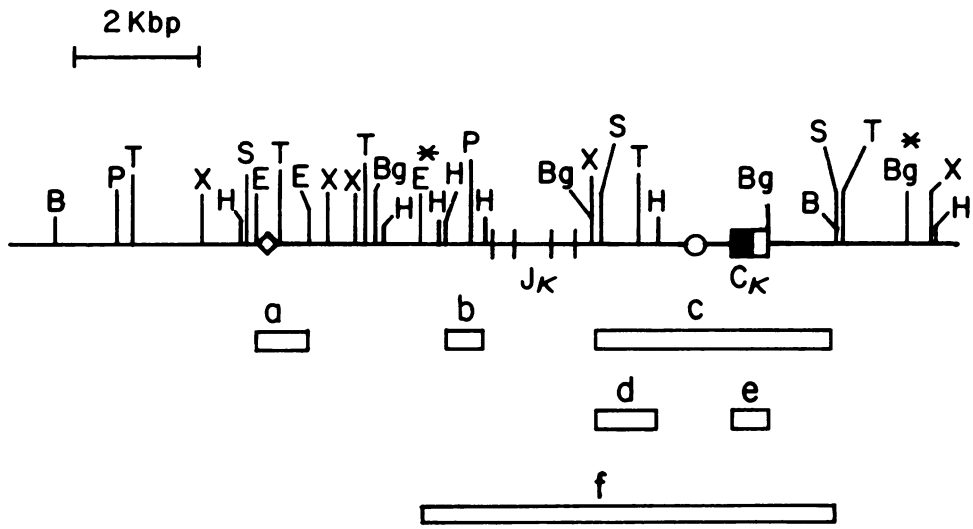
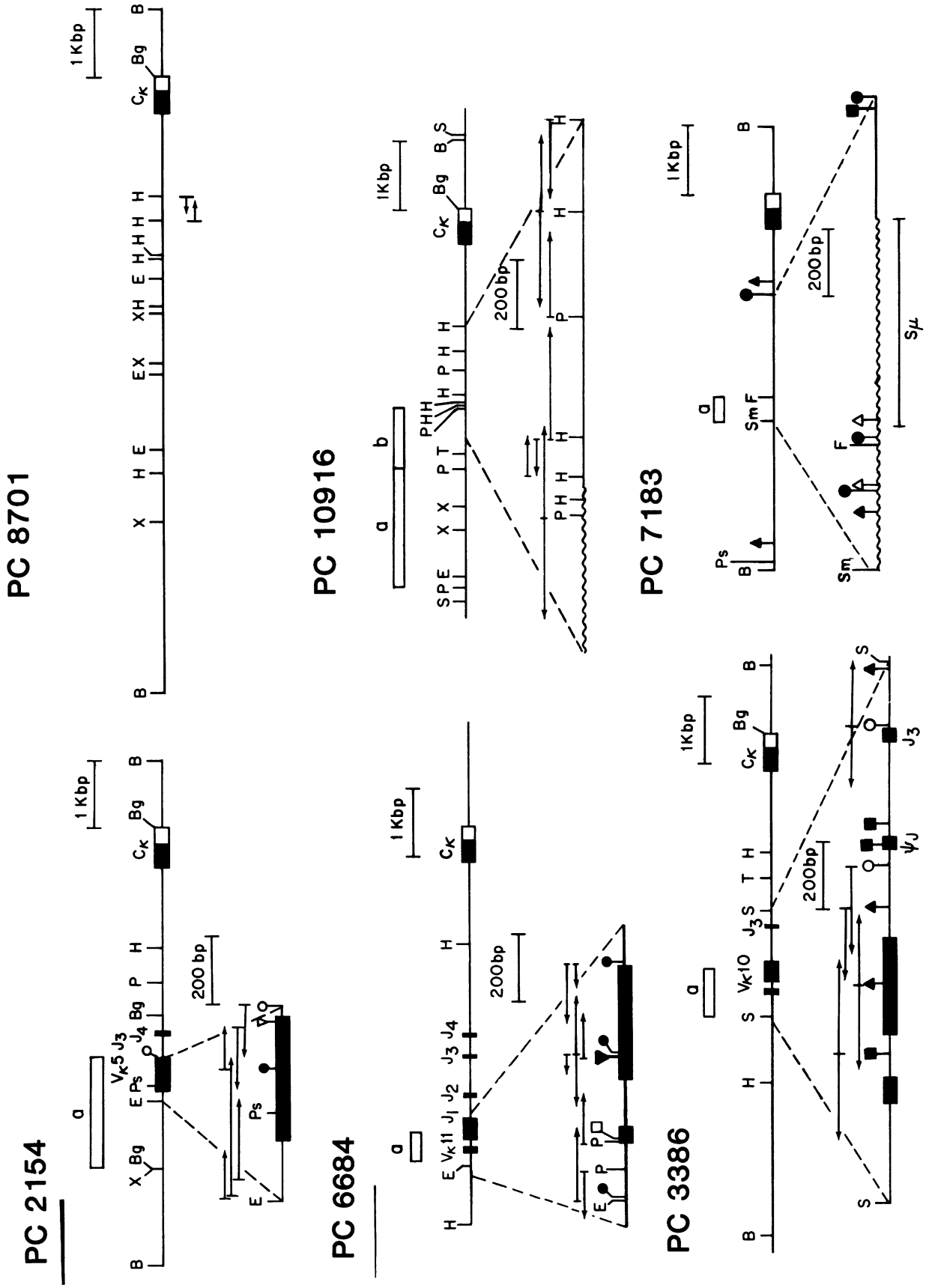
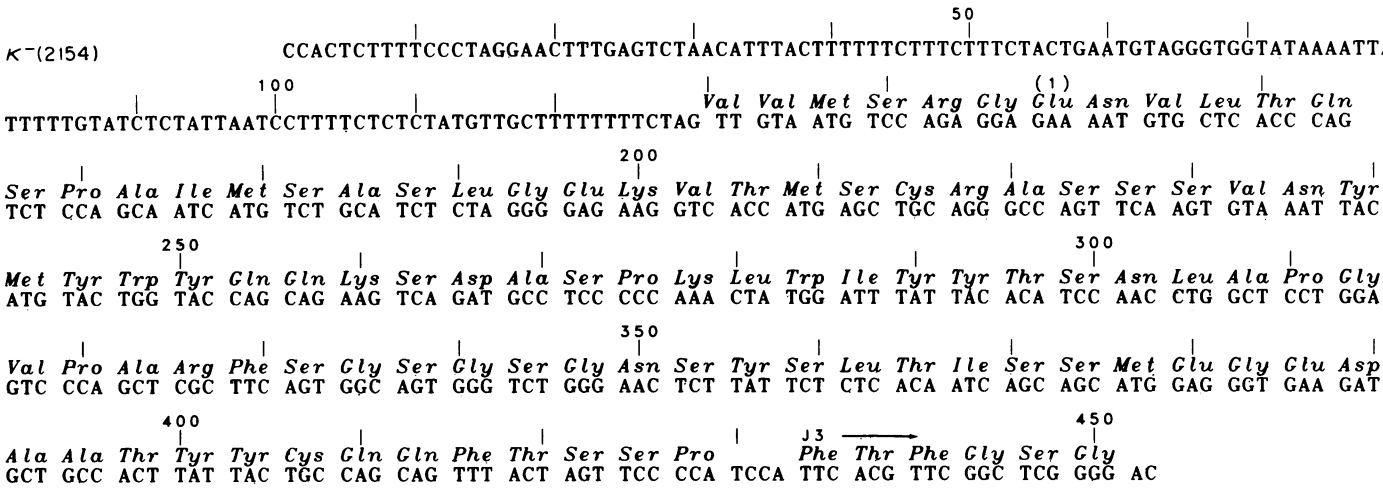


FIG. 1. Map of the κ locus in mouse germline DNA (κ^0 locus). Restriction sites: B, *Bam*HI; P, *Pvu*II; T, *Taq*I; X, *Xba*I; H, *Hin*dIII; S, *Sac*I; E, *Eco*RI; Bg, *Bgl*II. The locations of the C_κ exon (solid and open portions of the box denote coding and noncoding regions, respectively) and the four functional J_κ elements are shown, as are the locations of the enhancer (○) and pseudopromoter (◇) elements. Asterisks mark the ends of the known continuous sequence (32, 36; this paper). The sequence of the *Eco*RI fragment surrounding the pseudopromoter is also known (57). The various κ^0 probes used in this study are shown by the open rectangles a through f. κ^0(a), κ^0(d), κ^0(e), and κ^0(f) were cloned; κ^0(b) and κ^0(c) were purified from appropriate restriction enzyme digests. Kbp, Kilobase pairs.


PC 8701
PC 10916
PC 7183
PC 2154
PC 6684
PC 3386
1 Kbp
200 bp
200bp
Cκ
Bg
Sμ
Sm
SmF
Ps
Vκ5
Vκ11
Vκ10
Jκ
Jλ
Jμ
Jψ



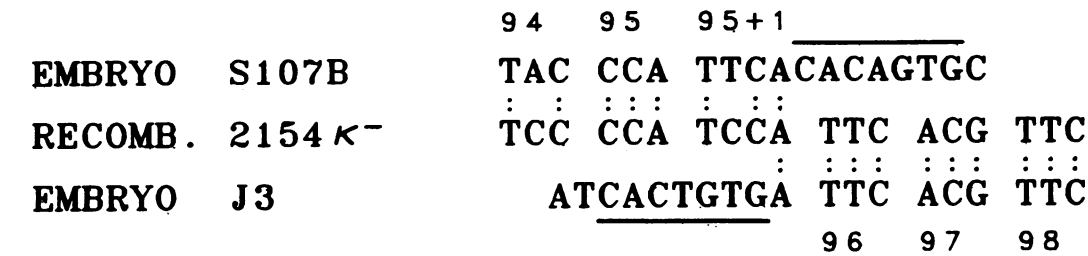


FIG. 3. Sequence of the κ⁻ allele of PC 2154. (Top) Translated sequence of V and $J_κ3$ regions, showing frameshift insertion at position 433. The N-terminal amino acid of the encoded κ chain is marked as (1). The $J_κ3$ sequence is translated as if there were no frame shift. (Bottom) Comparison of the sequence surrounding the frameshift insertion of the recombinant (recomb.) PC 2154 κ⁻ gene with the germline (embryo) sequence of a closely related $V_κ$ gene, S107B (26) and the germline $J_κ3$ sequence (32). Heptameric recombinase recognition signals are over- or underlined. Numbers refer to conventional V and J region codons.

(Table 1). Southern blot analysis with several restriction enzymes and a variety of probes from the $J_κ$-$C_κ$ region of the germline ($κ^0$) locus (Fig. 1) was used for preliminary characterization of the κ⁻ alleles. *Bam*HI digestion and hybridization with a probe such as $κ^0$(c) or $κ^0$(f) revealed two intense bands corresponding to the κ⁺ and κ⁻ alleles. The band representing the κ⁺ allele could usually be recognized because it was common to other PCs in which the same $V_κ21$ gene and $J_κ$ element were used (55), enabling us to identify the other band as the κ⁻ allele.

When *Bgl*II or *Xba*I DNA digests were hybridized to the $κ^0$(e) probe, two categories of results were obtained. For PCs 2154 and 6684, only a single 2.8-kilobase (kb) *Bgl*II fragment or 5.3-kb *Xba*I fragment was observed, indicating that in both κ⁺ and κ⁻ alleles the recombinational events were confined to the $J_κ$ region (Fig. 1) and consequently that germline organization was maintained downstream of the intronic *Bgl*II and *Xba*I sites. In contrast, for PCs 3386, 7183, 8701, and 10916, we observed fragments of novel sizes as well as germline-type *Bgl*II and *Xba*I fragments, suggesting that for these PCs the κ⁻ alleles may have been formed by abnormal rearrangements which altered the DNA in the region of the intronic *Bgl*II and *Xba*I sites. The sites of the κ⁻ recombinations were further localized by a similar Southern blot analysis of *Sac*I and *Hin*dIII digests. This analysis indicated that the germline *Sac*I site was abolished in the κ⁻ alleles of PCs 7183, 8701, and 10916 and that in the κ⁻ allele of PC 7183 the germline *Hin*dIII site was lost as well. Thus, κ⁻ recombinations were indicated to be in the $J_κ$ region for PCs 2154 and 6684, between the *Xba*I and *Sac*I sites in PC 3386, between the *Sac*I and *Hin*dIII sites in PCs 8701 and 10916, and 3′ of the *Hin*dIII site in PC 7183.

Based on the above information, DNA from each PC was digested with a suitable restriction enzyme and the appropriate fragment bearing the κ⁻ allele was enriched by gel electrophoresis. The various fragments were ligated to suitable λ phage vectors, packaged into lysogens, and cloned by conventional procedures. The cloned fragments, λ phage vectors, and probes used for screening are summarized in Table 1. In some cases we included a negative screen with a $V_κ21$ variable-region probe to ensure that we had not inadvertantly cloned the κ⁺-bearing fragment.

Each of the cloned κ⁻ genes was mapped with a battery of restriction enzymes and sequenced by the strategies shown in Fig. 2. The unique sequence structure of the PC 7183 κ⁻ allele has been reported in an earlier publication (56) and will

FIG. 2. Maps and sequence strategies for six cloned κ⁻ alleles. Restriction sites: Ps, *Pst*I; Sm, *Sma*I; F, *Fnu*DII; ○, *Acc*I; ●, *Hin*fI; ▽, *Ava*I; ▼, *Sfa*NI; □, *Dde*I; ■, *Ava*II; ▲, *Sau*3A; △, *Hpa*I. See Fig. 1 legend for other abbreviations. Probes specific for the particular κ⁻ alleles are shown by open rectangles. Sμ, μ switch region.

FIG. 4. Sequence of the κ⁻ allele of PC 6684. (Top) Sequence of V and $J_κ1$ (bracketed) regions, showing the putative T→C mutation at position 286 (*) which prevents splicing at the normal junction (↕), enabling recognition of a cryptic site (↑). The sequence is translated as if the normal splice site were used. Elements with presumed promoter function are underlined. CAP, Cap site. (Bottom) Translated sequence of the abnormally spliced transcript, showing the termination codon (***) which immediately precedes the splice junction (↑). *, T→C mutation.

only be summarized here. The sequence structures of the other κ⁻ alleles are presented in Fig. 3 through 6 and discussed in the following section.

Sequence structure of κ⁻ alleles. (i) PC 7183. The κ⁻ gene of PC 7183 (Fig. 2) was created by a translocation between chromosomes 15 and 6 (the κ-bearing chromosome), recombination at the κ locus occurring 20 nucleotides 3′ of the aforementioned *Hin*dIII site. Between the chromosome 15 region and the κ region there is a segment of about 700 nucleotides which contains sequences resembling those of the μ switch region; the origin and mechanism of insertion of this segment are unclear (51). In this κ⁻ gene the region of the κ locus that has been shown to have enhancer activity (42, 44) is still intact and is located about 450 to 650 nucleotides downstream of the recombination site.

(ii) PC 2154. The sequence of the PC 2154 κ⁻ allele (Fig. 3) revealed that it was created by recombination between a variable-region gene of the $V_κ5$ group and the $J_κ3$ element. The group classification of this and other variable-region genes involved in these κ⁻ rearrangements was ascertained by comparing the encoded amino acid sequence with a compilation of other mouse κ chains (23). The cause of nonproductivity was found to be a 1-nucleotide frame shift at the VJ junction which causes premature termination of the encoded κ chain. Such frameshift lesions in κ⁻ alleles have been noted previously (3, 7, 58). Interestingly, the $V_κ5$ gene used in this recombination was similar but not identical to S107B, a V gene implicated in an abnormal κ rearrangement in PC S107 (7, 26). S107B has longer-than-normal spacing between the last V codon (codon number 95) and the heptameric recombination signal (Fig. 3), a feature which may make it more prone to imprecise recombinations (26).

(iii) PC 6684. The κ⁻ allele in PC 6684 was produced by a perfectly normal recombination between a variable-region gene of the $V_κ11$ group and $J_κ1$ (Fig. 4). A search for the cause of nonproductivity revealed only one plausible lesion, an improper 5′ splice junction in the intron that interrupts the signal peptide-encoding sequence. This intron begins with a GC (nucleotides 285 and 286) rather than the usual GT (8). As will be demonstrated below, this lesion causes the utilization of a cryptic splice site (between nucleotides 307 and 308), which is preceded by a termination codon (Fig. 4). Inspection of the 5′ flank of the $V_κ11$ gene revealed the ATTTGCAT and TATA sequences that are believed to contribute to the promoter function of $V_κ$ genes (6, 13, 25, 37). The position of these sequences suggests that the cap site of this gene should be only a few nucleotides upstream of the ATG initiation codon; this is indeed the case, as verified by S1 nuclease protection experiments (see below).

(iv) PC 3386. The sequence of the κ⁻ gene in PC 3386

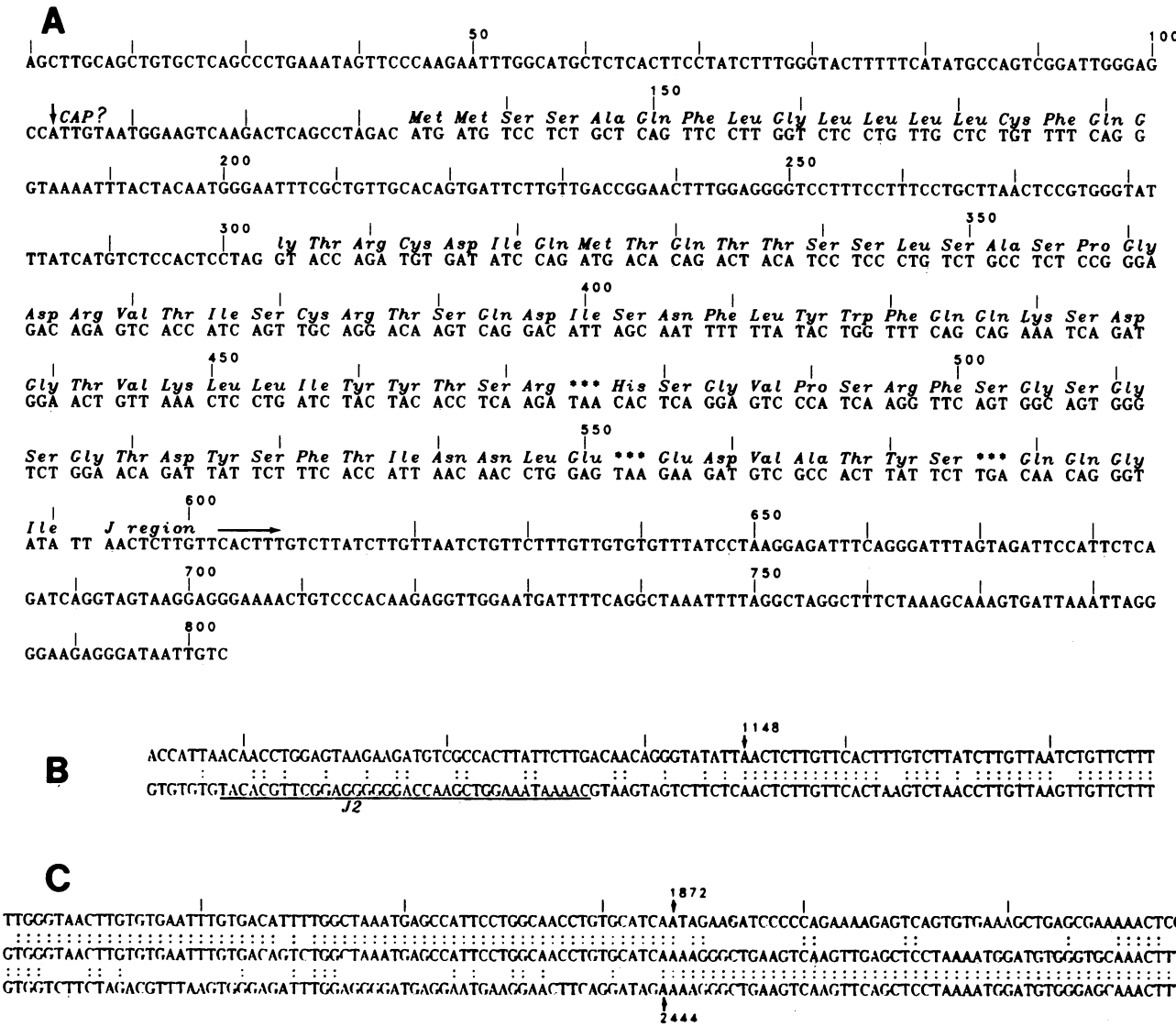


FIG. 5. Sequence of the κ⁻ allele of PC 3386. (A) Translated sequence of the VJ region, showing location of three termination codons (***) and the junction with the 3′ flank of the $J_κ2$ element at position 591. CAP, Cap site. (B) Comparison of the PC 3386 κ⁻ gene (top line) with the germline $J_κ2$ ($κ^0$) region (bottom line). The recombination point is at position 1148; the coding portion of $J_κ2$ is underlined. (C) Comparison of the PC 3386 κ⁻ sequence with selected portions of the $κ^0$ sequence, illustrating the deletion between positions 1871 (or 1872) and 2444 (or 2445). Top line, $κ^0$ 5′; center line, κ⁻; bottom line, $κ^0$ 3′. Numbering in B and C is according to Max et al. (32).

indicated that its formation involved several independent recombinational events (Fig. 5). In the 5′ region there was a variable gene of the $V_κ10$ group joined to a site 16 base pairs (bp) downstream of the $J_κ2$ element (Fig. 5B). This V gene has multiple defects: it contains three termination codons and has only 92 codons instead of the usual 95 (Fig. 5A). This unusual rearrangement may have resulted from two sequential events: recombination between a $V_κ10$ gene and either $J_κ1$ or $J_κ2$ and subsequent deletion of a segment containing the missing V and J sequences. In addition, this κ⁻ allele incurred a deletion which removed a 573-bp region containing the $J_κ4$ element and the previously mentioned *Bgl*II and *Xba*I sites (Fig. 5C). Aligning the sequences surrounding these recombination sites with their germline counterparts (Fig. 5B and C) did not reveal any extensive homology, suggesting that the deletion(s) probably occurred by a mechanism that does not require homologous pairing. Another interesting feature of this κ⁻ gene was the relatively large number of somatic mutations in the vicinity of the recombination sites. Within the first 100 nucleotides 3′ of the recombination site, the substitutions amounted to 12%, which was significantly higher than that observed for most $κ^+$ alleles (less than 3% [K. E. Huppi, Ph.D. thesis, University of Pennsylvania, Philadelphia, 1984]).

(v) PC 8701 and PC 10916. The κ⁻ alleles of the two independently derived PCs 8701 and 10916 have a common feature, a 2,462-bp deletion which extends from the heptameric recombination signal associated with $J_κ1$ to a similar isolated heptamer located 1,100 nucleotides 3′ of $J_κ4$ (Fig. 6A). This same isolated heptamer was previously found to be involved in the κ⁻ rearrangement of the MPC11 PC; however, in MPC11 it recombines with the heptameric recognition signal of a V gene rather than that of a $J_κ$ element (48). Moreover, in MPC11 only a single heptamer remains at the recombination site, consistent with the possibility of a homologous pairing mechanism (48), whereas in PCs 8701

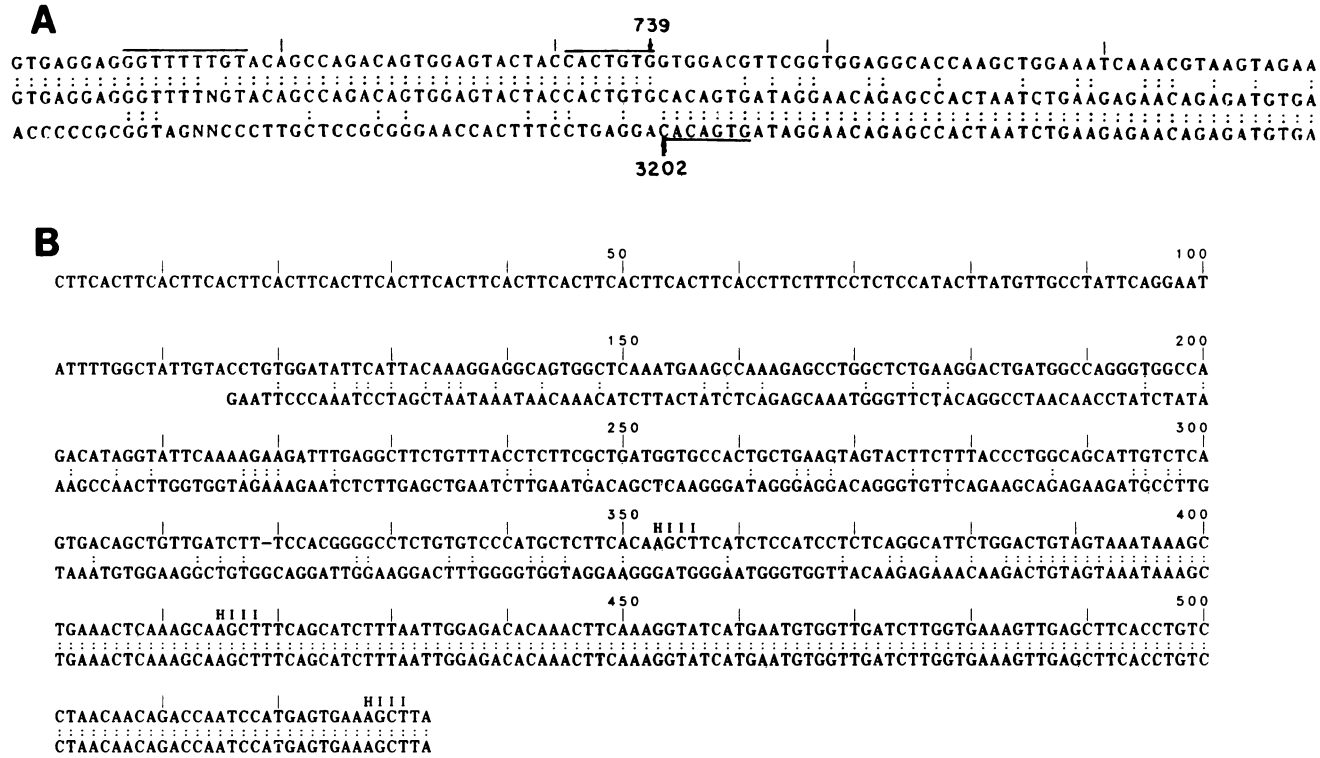


FIG. 6. Sequences of the κ^- alleles of PCs 8701 and 10916. (A) Sequence spanning the 2,462-bp deletion common to PC 8701 and PC 10916 compared with the germline (κ^0) sequences surrounding the deletion endpoints (arrows). Heptamer and nonamer recognition signals are over- or underlined. Numbering is according to Max et al. (32). Top line, κ^0 5'; center line, κ^-; bottom line, κ^0 3'. (B) Sequence of the upstream portion of the PC 10916 κ^- allele (top lines) compared with the κ^0 sequence (bottom lines) in the region surrounding the breakpoint at position 382. The locations of the *Hin*dIII (HIII) sites are shown; the site at position 527 marks the 5' end of the previously published κ^0 sequence (32).

and 10916 two complete heptamers remain at the recombination site, consistent with a conventional VJ recombinational mechanism (see below).

In addition, PC 10916 underwent another recombination 886 nucleotides upstream of the deletion (Fig. 6B). This recombination replaced the upstream region of the κ locus, including the pseudopromoter (cf. Fig. 1), with DNA that did not bear any resemblance to a V gene. This DNA sequence is unique in the mouse genome, as evidenced by the simple pattern observed when a portion of it [probe κ^-10916(b), Fig. 2] was hybridized to a Southern blot of *Eco*RI-digested germline (NZB mouse liver) and PC 10916 DNA. The germline DNA gave only a single band, and the PC DNA gave one rearranged and one unrearranged fragment of roughly half intensity (Fig. 7). A similar analysis of a κ^+/κ^0 PC, 3741, revealed only the germline *Eco*RI fragment (data not shown). The chromosomal location of this foreign DNA is unknown, although by analogy to PC 7183, it is reasonable to suppose that it was joined to the κ locus by a chromosomal translocation. A comparison of its sequence with that of the germline (κ^0) sequence over a 266-nucleotide stretch upstream of the recombination point did not reveal any significant homology which could account for the recombination specificity.

Expression of κ^- alleles that contain V genes. All of the κ^- alleles that contained V genes were actively transcribed. A Northern blot analysis of poly(A)$^+$ nuclear RNA from PCs 2154, 6684, and 3386 revealed discrete primary transcripts

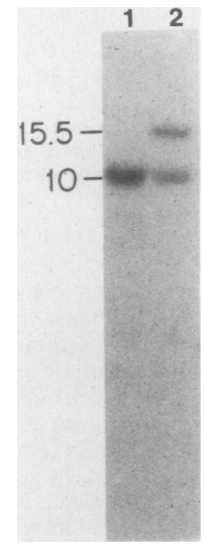


FIG. 7. Southern blot analysis of the κ^- allele in PC 10916: uniqueness of germline sequence. DNAs (10 μg) from an NZB mouse liver (lane 1) and PC 10916 (lane 2) were digested with *Eco*RI, separated on a 0.8% agarose gel, blotted onto nitrocellulose, and hybridized with the κ^-10916(b) probe. The approximate sizes of the *Eco*RI fragments (in kilobases) are shown.

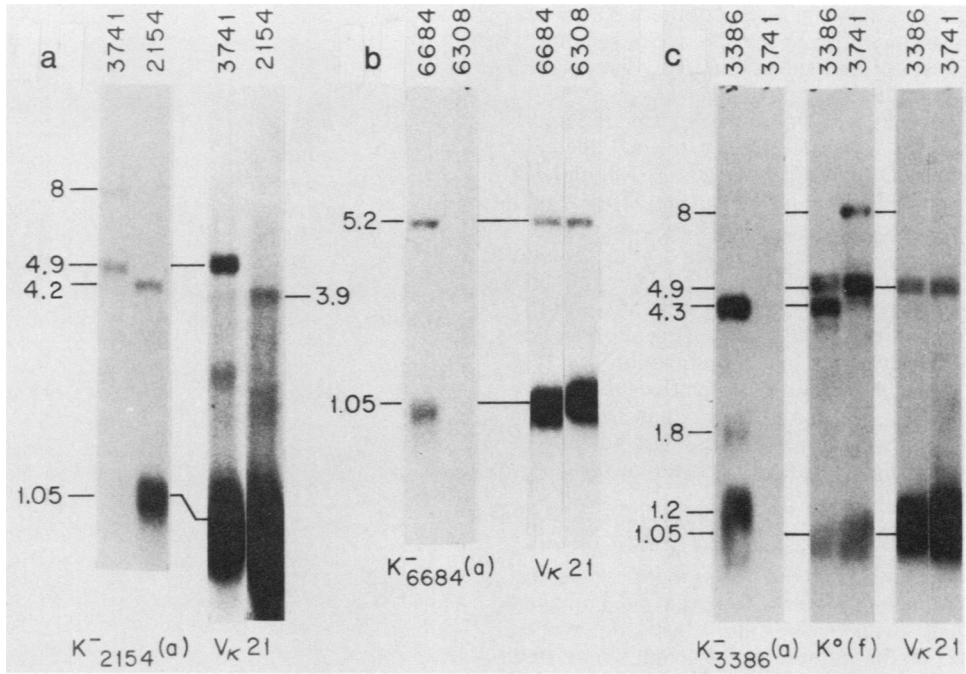


FIG. 8. Expression of V gene-containing κ^- alleles as determined by Northern blot analyses of nuclear RNA. Samples of poly(A)$^+$ nuclear RNA (5 μg) from PCs 6684, 3386, 3741, and 6308 and 10-μg samples from PC 2154 were size fractionated by electrophoresis on 1% agarose gels containing formaldehyde, transferred to nitrocellulose, and hybridized with the probes indicated below the lanes. The $V_\kappa 21$ and κ^0(f) probes were cloned recombinant plasmids: $V_\kappa 21$ contained sequences of the V_κ region expressed in MOPC321 (41), and κ^0(f) contained the J_κ-C_κ gemline region (Fig. 1). The κ^- probes were the purified restriction fragments shown in Fig. 2. The approximate sizes (in kilobases) of the various RNA components are indicated. Autoradiograms were exposed with an intensifier screen at −70°C as follows: (a) κ^-2154(a), 16 h; $V_\kappa 21$, 24 h; (b) κ^-6684(a), 0.5 h; $V_\kappa 21$, 19 h; (c) κ^-3386(a), 16 h; κ^0(f), 1.5 h; and $V_\kappa 21$, 22 h.

and processed derivatives appropriate to the gene arrangements of the κ^+ and κ^- alleles (Fig. 8). Each RNA sample was hybridized with a probe derived from the cloned κ^- allele and with a probe specific for the κ^+ allele (a cloned $V_\kappa 21$ cDNA fragment that reacted with all members of the $V_\kappa 21$ family). PC 3386 RNA was also hybridized with probe κ^0(f), which spans the J_κ-C_κ region. To verify the specificity of the hybridization probes, we included poly(A)$^+$ nuclear RNA samples from the $V_\kappa 21$-expressing PCs 3741 and 6308, which had κ^+/κ^0 genotypes (Table 1) and produced RNAs of predictable size and sequence content.

(i) PC 2154. In PC 2154 the κ^- allele, a V→J_3 rearrangement, produced a 4.2-kb primary transcript, whereas the κ^+ allele, a V→J_4 rearrangement, produced a 3.9-kb primary transcript (Fig. 8a). Both transcripts were processed into 1.05-kb mature κ mRNAs. Since the κ^-2154(a) probe contained the $J_\kappa 3$ element, it also hybridized to the 4.9-kb primary transcript of the κ^+ allele (V→J_2) and the 8-kb transcript of the κ^0 allele in PC 3741.

(ii) PC 6684. Both the κ^- and κ^+ alleles of PC 6684 were V→J_1 rearrangements and thus both produced 5.2-kb primary transcripts as well as 1.05-kb processed derivatives (Fig. 8b). Although the resolution afforded by Northern blot analysis was insufficient to detect any aberration in the splicing of the first intron, this was readily detected by S1 nuclease protection experiments.

The S1 nuclease protection experiments (Fig. 9) provided information on the location of the cap site of the $V_\kappa 11$ gene and on the splice junctions used in the excision of intron 1. For determination of the cap site and the 3′ splice junction, poly(A)$^+$ nuclear RNA from PC 6684 was annealed with either a 5′-end-labeled 263-bp *Pvu*II-*Hin*fI fragment or a similarly labeled 429-bp *Hin*fI fragment under conditions that favored RNA-DNA hybridization. After S1 nuclease digestion, both fragments yielded large amounts of a 67-nucleotide S1-resistant product and smaller amounts of a 261-nucleotide product (Fig. 9a, lanes 2 and 5). The 67-nucleotide product corresponds to the region of exon 2 that would be protected by the 1.05-kb processed RNA component if the proper 3′ splice junction of intron 1 were used. The 261-nucleotide product corresponds to the 5′ region of a primary transcript in which the cap site is located 2 nucleotides from the *Pvu*II site, i.e., only 4 nucleotides upstream from the translational start codon (Fig. 4). The fully protected 263- and 429-nucleotide products were generated by the small amount of DNA reannealing that occurred under these conditions, as seen by controls in which *Saccharomyces cerevisiae* RNA was substituted for the poly(A)$^+$ nuclear RNA (Fig. 9a, lanes 3 and 6).

For characterization of the 5′ splice junction, we annealed poly(A)$^+$ nuclear RNA with a 3′-end-labeled 245-nucleotide antistrand derived from a *Dde*I-*Hin*fI fragment that terminates in exon 1. If the conventional splice junction bearing the GU→GC mutation were used, we would expect to observe a 36-nucleotide S1-resistant product (Fig. 9b). However, what we observed after S1 digestion at 20°C (lane 3) and 37°C (lane 4) was a series of fragments about 50 to 60 nucleotides long. Inspection of the appropriate region of the PC 6684 κ^- sequence revealed a plausible 5′ splice junction (between nucleotides 307 and 308, Fig. 4), the use of which generated an RNA that would yield a 59-nucleotide protected fragment. This splice junction was preceded by an AT-rich stretch that would be particularly vulnerable to S1 nuclease nibbling; this could account for the heterogeneous

array of protected fragments. The results of these S1 protection experiments confirmed the abnormal mode of splicing of the PC 6684 κ^- transcript and thus definitively established the cause of the nonproductivity of this gene.

(iii) PC 3386. In PC 3386 a 4.9-kb primary transcript and a 1.05-kb mature mRNA are produced by the κ^+ allele (a $V \rightarrow J_2$ rearrangement), and components of 4.3, 1.8, and 1.2 kb are produced by the κ^- allele (Fig. 8c). The 4.3-kb component is of the appropriate size for a transcript initiated at the putative cap site of the $V_\kappa 10$ gene (Fig. 5) and terminated at the C_κ polyadenylation site. The 1.8- and 1.2-kb components are probably processed derivatives of the 4.3-kb transcript formed by the utilization of cryptic 5′ splice junctions located downstream of the recombination site. It is noteworthy that the ratio of processed derivatives to primary transcript is significantly lower for the κ^- than for the κ^+ RNA. This suggests either a lower processing efficiency of the κ^- transcript or a higher rate of turnover of the aberrantly processed products.

In all three PCs of this category, the nuclear content of the κ^- primary transcript is greater than or equal to that of the κ^+ primary transcript. For PC 6684, a comparison of the autoradiographic exposures required to reveal 5.2-kb bands of equivalent intensity with the κ^+- and κ^--specific probes and a consideration of the relative probe lengths indicated that the κ^- primary transcripts are at least an order of magnitude more abundant than the κ^+ primary transcripts. For PC 3386 the 4.3-kb κ^- transcript is about four times more abundant than the 4.9-kb κ^+ transcript, as judged by comparing blots hybridized with the κ^- and κ^+ probes and by comparing the intensity of the 4.3- and 4.9-kb components in the blot hybridized with the κ^0(f) probe. As will be demonstrated later, the difference in primary transcript content in PC 3386 is paralleled by a similar difference in transcription rate between the κ^- and κ^+ alleles.

Expression of κ^- alleles that lack V genes. Northern blot analysis of poly(A)$^+$ nuclear RNA from the PCs with κ^- alleles that lack V genes was carried out with probes representing selected regions of the κ^0 locus, the $V_\kappa 21$ gene, and upstream portions of the κ^- genes (Fig. 10). All three of these κ^- genes are transcribed; however, the content of the κ^- transcripts is very low compared with that of κ^+ transcripts in the same nuclei or with that of 8-kb nuclear transcripts in PCs bearing κ^0 alleles.

(i) PC 8701. In PC 8701 the κ^+ allele (a $V \rightarrow J_3$ rearrangement) produces a 4.2-kb transcript which is processed to a 1.05-kb mature mRNA component, and the κ^- allele produces a 5.5-kb transcript and a 1.2-kb component, which is presumably a processed derivative. For the 5.5-kb transcript, the relative hybridization with the κ^0(a) and κ^0(b) probes (Fig. 1) was the same as that of the 8-kb transcript of the κ^0 allele in the control PC 3741 (Fig. 10a and b). Thus, the size and sequence characteristics of the 5.5-kb transcript suggest that it is simply a counterpart of the 8-kb κ^0 transcript which bears the 2,462-bp deletion region of this κ^- allele (Fig. 6A). As judged from the band intensities, the 5.5-kb κ^- transcript of PC 8701 is only about 10 to 20% as abundant as the 8-kb κ^0 transcript of PC 3741. The fact that these two RNA samples contained essentially the same amount of κ^+ transcripts (Fig. 10d) shows that the low κ^-/κ^0 abundance ratio was not due to a difference in the quality or quantity of RNA analyzed. The 5.5- and 8-kb transcripts may have been processed into similar 1.2-kb derivatives that retained 5′-terminal sequences [detectable as faint bands with the κ^0(a) probe]. However, differences in the breakdown pathways for the two transcripts were also apparent.

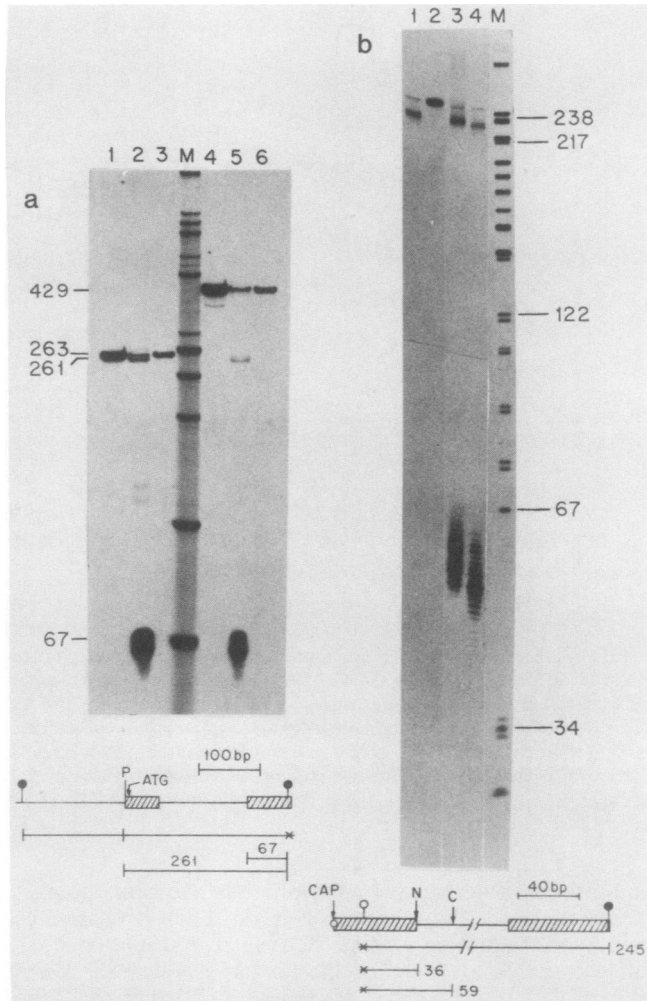


FIG. 9. Determination of the splice junctions and cap site (CAP) of the RNA produced by the κ^- allele of PC 6684. (a) 3′ splice junction and cap site. Samples (8 μg) of poly(A)$^+$ nuclear RNA from PC 6684 (lanes 2 and 5) or *S. cerevisiae* RNA (lanes 1, 3, 4, and 6) were hybridized with 8 ng of either a 263-bp 5′-end-labeled *Pvu*II (P)-*Hin*fI (●) fragment (lanes 1 through 3) or a 429-bp 5′-end-labeled *Hin*fI fragment (lanes 4 through 6) for 3 h at 45°C in a solution containing 80% formamide and 0.4 M NaCl. The hybridization mixtures were diluted, incubated for 30 min at 37°C with (lanes 2, 3, 5, and 6) or without (lanes 1 and 4) 4,000 U of S1 nuclease (Miles Pharmaceuticals), ethanol precipitated, and electrophoresed on a 5% polyacrylamide–7 M urea gel together with a set of markers (lane M) prepared from an *Hae*III digest of ϕX174. The diagram shows the 67-nucleotide protected fragment which defines the 3′ splice junction and the 261-nucleotide protected fragment which defines the cap site. Fragment sizes (in nucleotides) are indicated. Hatched areas are exons. (b) 5′ splice junction. Samples (5 μg) of poly(A)$^+$ nuclear RNA from PC 6684 (lanes 3 and 4) or *S. cerevisiae* RNA (lanes 1 and 2) were hybridized for 3 h at 45°C in a solution containing 50% formamide and 0.75 M NaCl to about 5 pmol of a 3′-end-labeled minus strand of a 245-bp *Dde*I (○)-*Hin*fI (●) fragment which spans the first intron. The hybridization mixtures were diluted and either not treated further (lane 2) or incubated for 30 min at 37 (lanes 1 and 4) or 20°C (lane 3) with 1,000 U of S1 nuclease and analyzed on a 6% polyacrylamide–7 M urea gel together with a set of markers (lane M) prepared from an *Hpa*II digest of AFP-pBR322 recombinant clone 1303, provided by S. Tilghman. The diagram shows the 36- and 59-nucleotide fragments that would be observed, depending on whether the normal (N) or a cryptic (C) splice site was used. Fragment sizes (in nucleotides) are indicated.

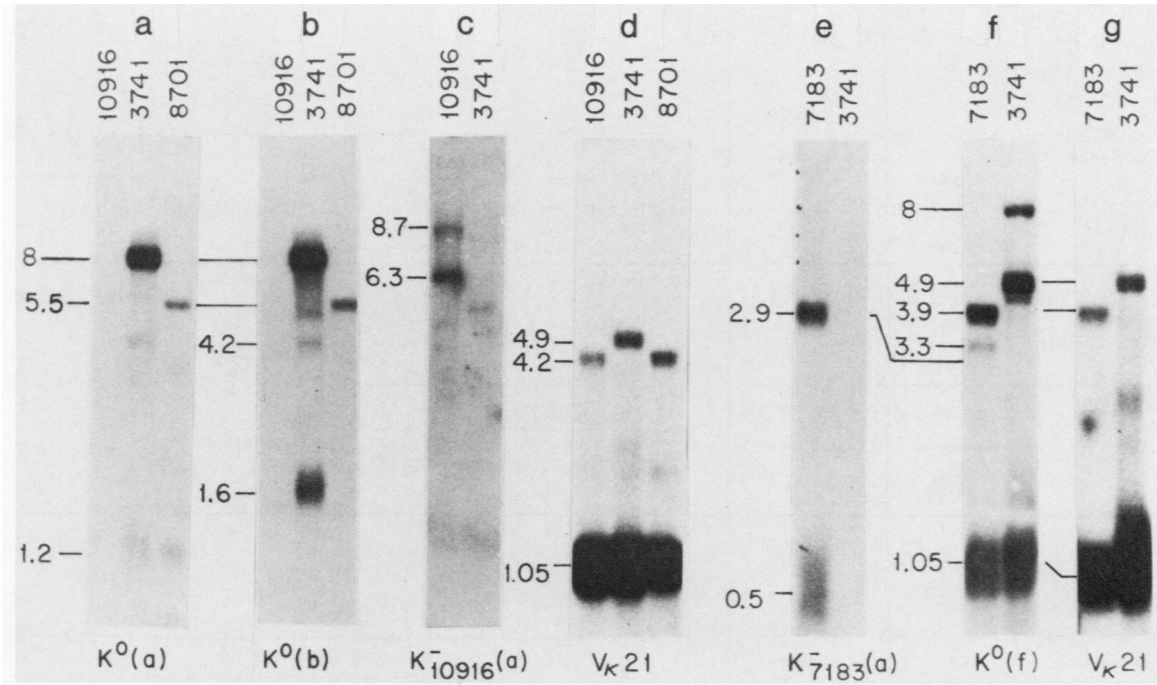


FIG. 10. Expression of V gene-lacking κ^- alleles as determined by Northern blot analysis of nuclear RNA. Samples (5 μg) of poly(A)$^+$ nuclear RNA from PCs 8701, 10916, 7183, and 3741 were analyzed as described in the legend to Fig. 8. Probes κ^0(a), κ^0(f), κ^-7183(a), and V_κ21 were cloned recombinant plasmids; probes κ^0(b) and κ^-10916(a) were purified restriction fragments. Autoradiographic exposure times at −70°C with intensifier screens were (a) 23 h, (b) 4.5 h, (c) 14 days, (d) 24 h, (e) 10 days, (f) 6 h, and (g) 24 h. When the blot in panel b was exposed for 3 days or longer, discrete 8.7- and 6.3-kb components were clearly visible in the PC 10916 lane. The very faint bands seen in panel c are due to a slight contamination of the κ^-10916(a) probe with a 3′ *Pvu*II fragment which hybridized to κ^+ and κ^0 transcripts. Probes used are indicated below each panel; fragment sizes (in kilobases) are indicated.

For example, a 1.6-kb RNA component containing κ^0(b) sequences and lacking κ^0(a) sequences is present in PC 3741 but clearly absent in PC 8701.

(ii) PC 10916. In PC 10916 the κ^+ allele (a V→J_3 rearrangement) produces a 4.2-kb transcript (Fig. 10d). No poly(A)$^+$ RNA components were detected with the κ^0(a) probe (Fig. 10a). Although the sequence recognized by this probe is absent from both the κ^+ and κ^- alleles, it persists at another location in the PC 10916 genome (55), possibly as part of a reciprocal translocation involved in the generation of the κ^- allele. The absence of RNA components containing κ^0(a) sequences and the corresponding transcriptional data (Fig. 11) demonstrate that these rearranged κ^0(a) sequences (which contain the germline pseudopromoter) are transcriptionally inert. In contrast, the foreign DNA that recombined into the κ^- locus of PC 10916 is transcriptionally active, although the accumulation of κ^- transcripts is very low compared with that of κ^+ and κ^0 transcripts. With prolonged exposure of the autoradiograms, two poly(A)$^+$ transcripts, of 8.7 and 6.3 kb, were revealed with the κ^-10916(a) probe (Fig. 10c), the κ^-10916(b) probe, and the κ^0(b) probe (data not shown). The size of these κ^- transcripts and their pattern of probe reactivity suggest that they originate in the foreign DNA segment and extend into the κ locus. Their accumulation is only about 2 to 3% of that of the κ^+ transcripts, as judged by the relative exposure times needed to achieve comparable band intensities and a consideration of relative probe lengths (cf. Fig. 10c and d). Transcripts from the foreign DNA segment were not detected in the poly(A)$^+$ nuclear RNA of PC 3741, indicating that this region of DNA is not transcribed in PCs when it is at its normal chromosomal location.

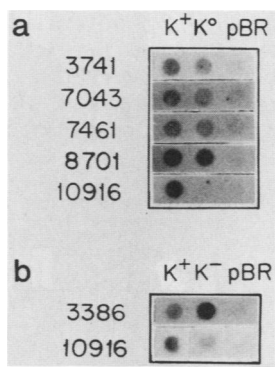


FIG. 11. Relative transcriptional activity of κ^+, κ^0, and κ^- alleles as determined by transcriptional run-on experiments. RNA isolated from about 10^7 PC nuclei that had been incubated for 10 min with [α-^{32}P]UTP was hybridized with nitrocellulose strips containing 4-μg dots of linearized recombinant plasmid DNA bearing the following inserts: κ^+, V_κ21; κ^0, κ^0(a); κ^-, κ^-3386(a) or κ^-10916(a); pBR, none (pBR322 DNA only).

(iii) PC 7183. The expression of κ genes in PC 7183 is similar to that in PC 10916. The κ^+ allele, a V→J_4 rearrangement, produces a 3.9-kb transcript that was revealed by the V_κ21 and κ^0(f) probes (Fig. 10), whereas the translocated chromosome 15 portion of the κ^- allele, monitored with the κ^-7183(a) probe, produces a 2.9-kb transcript, a 0.5-kb component, and trace quantities of larger components ranging from 4 to 7 kb (Fig. 10e). The amount of the 2.9-kb κ^- transcript is about 10% of that of the PC 3741 κ^0 transcript. Since this κ^- transcript could be clearly resolved from the

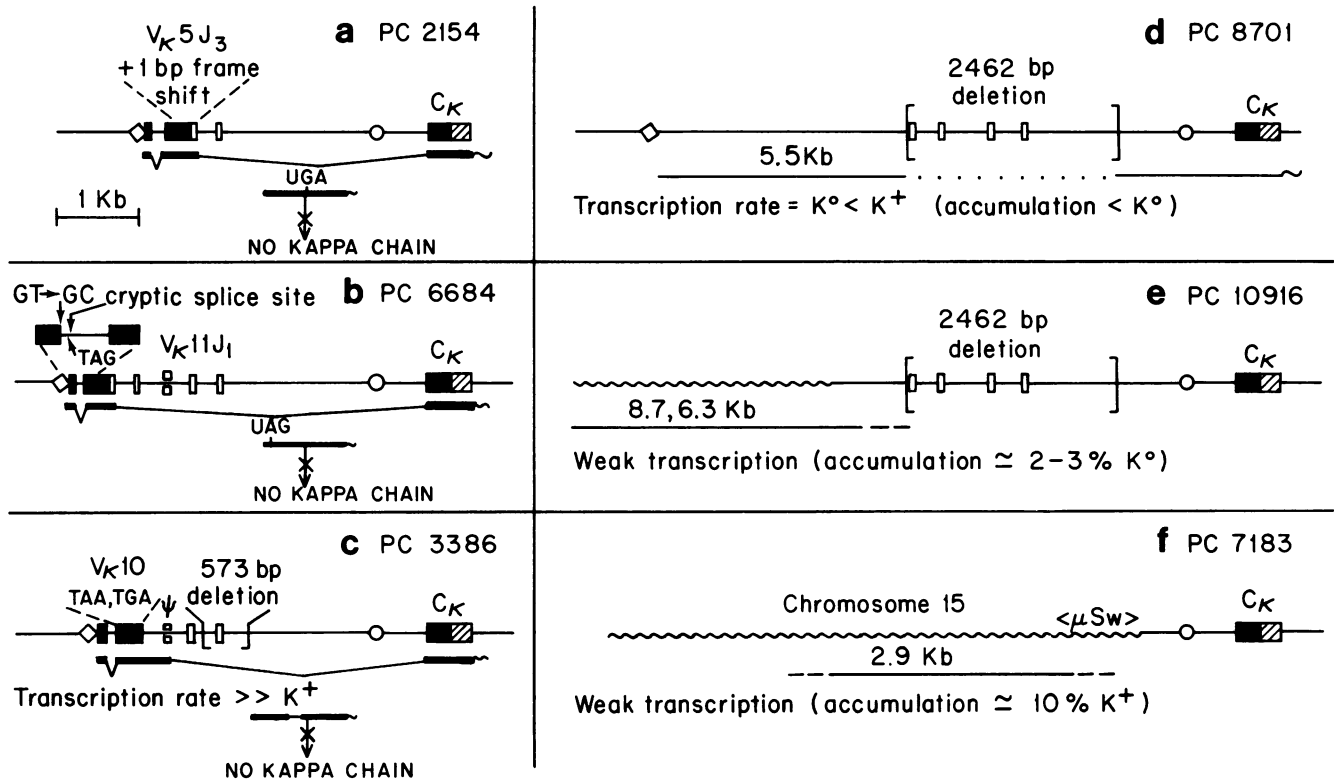


FIG. 12. Schematic representation of the lesions in the six κ^- alleles under study and their consequences for κ-chain expression. The genes (top) and transcripts (bottom) are diagrammed, with the relevant exons shown as boxes and the introns shown as lines. ○, Enhancer element; ◇, promoter or pseudopromoter element. The pseudo J_κ element (ψ) is shown as a box broken above and below the line in panels b and c but is omitted in panels d and e. Deleted regions in c, d, and e are bracketed and included in the diagram although they are absent from the genes. Foreign (non-κ) DNA is shown by a wavy line in e and f. μSw, μ switch region. The processed mRNAs and locations of translational termination codons are also shown in panels a, b, and c, although the precise locations of the splice junctions used in the aberrant processing of the κ^-3386 transcript are not known.

3.3-kb partially spliced derivative of the κ^+ transcript (41), we could investigate whether it hybridized to the κ^0(f) probe. Even after a lengthy exposure of the autoradiogram, the 2.9-kb component was not revealed by this probe, indicating that it does not extend into the κ region of the PC 7183 recombinant gene. In PC 3741, no poly(A)$^+$ RNA transcripts were detected with the κ^-7183(a) probe, indicating that this region of chromosome 15 is not transcribed normally in the PCs.

Comparative transcription rates of κ^0, κ^+, and κ^- genes. The differences in the amount of nuclear RNA produced by κ^- alleles and by κ^0 and κ^+ alleles could be attributable to differences in the rate of transcription, the rate of turnover, or both. To distinguish between these possibilities, we made direct measurements of the transcription rate of the κ alleles by the nuclear run-on method (17, 30, 34), in which growing RNA chains were briefly labeled in isolated nuclei and then hybridized to immobilized DNA representing selected regions of the κ^+, κ^0, and κ^- genes. Three PCs from the κ^+/κ^- series (PCs 3386, 8701, and 10916) were examined and compared with three PCs possessing the κ^+/κ^0 genotypes (PCs 3741, 7043, and 7461 [Table 1]). Transcription was measured with the following recombinant plasmids, immobilized as dots on nitrocellulose strips: V_κ21 for the κ^+ alleles, κ^0(a) for the κ^0 and appropriate κ^- alleles, κ^-3386(a) for the κ^- allele of PC 3386, and κ^-10916(a) for the κ^- allele of PC 10916. The hybridizable sequences, in these DNA probes are approximately 300, 800, 350, and 1,700 nucleotides long, respectively. Equivalent samples of pBR322 DNA were used to assess nonspecific background. The DNA was in large excess over the amount of hybridizable RNA so that the hybridization signal was directly proportional to the RNA input (data not shown). Under these conditions the measurements were not affected by differences in the specific radioactivity of the hybridizing species. Relative transcription rates were calculated from the relative dot intensity divided by the relative length of the hybridizable DNA sequence in the dots.

The results of these experiments (Fig. 11, Table 2) were remarkably uniform. In the three κ^+/κ^0 PCs 3741, 7043, and 7461, the transcription rate of the κ^0 allele is 1/4 to 1/5 that of

TABLE 2. Relative transcription rates of κ^+, κ^0, and κ^- alleles

PC	Relative rate of transcription[a]	
	κ^0/κ^+	κ^-/κ^-
3741	0.20	
7043	0.21	
7641	0.27	
8701		0.34
10916		0.03
3386		3–5

[a] Data were obtained from densitometric scans of the experiment shown in Fig. 11. Values represent the ratio of peak heights divided by the ratio of hybridizable sequence (see the text). Similar data were obtained in replicate experiments. The data for PC 3386 represent the range for three experiments.

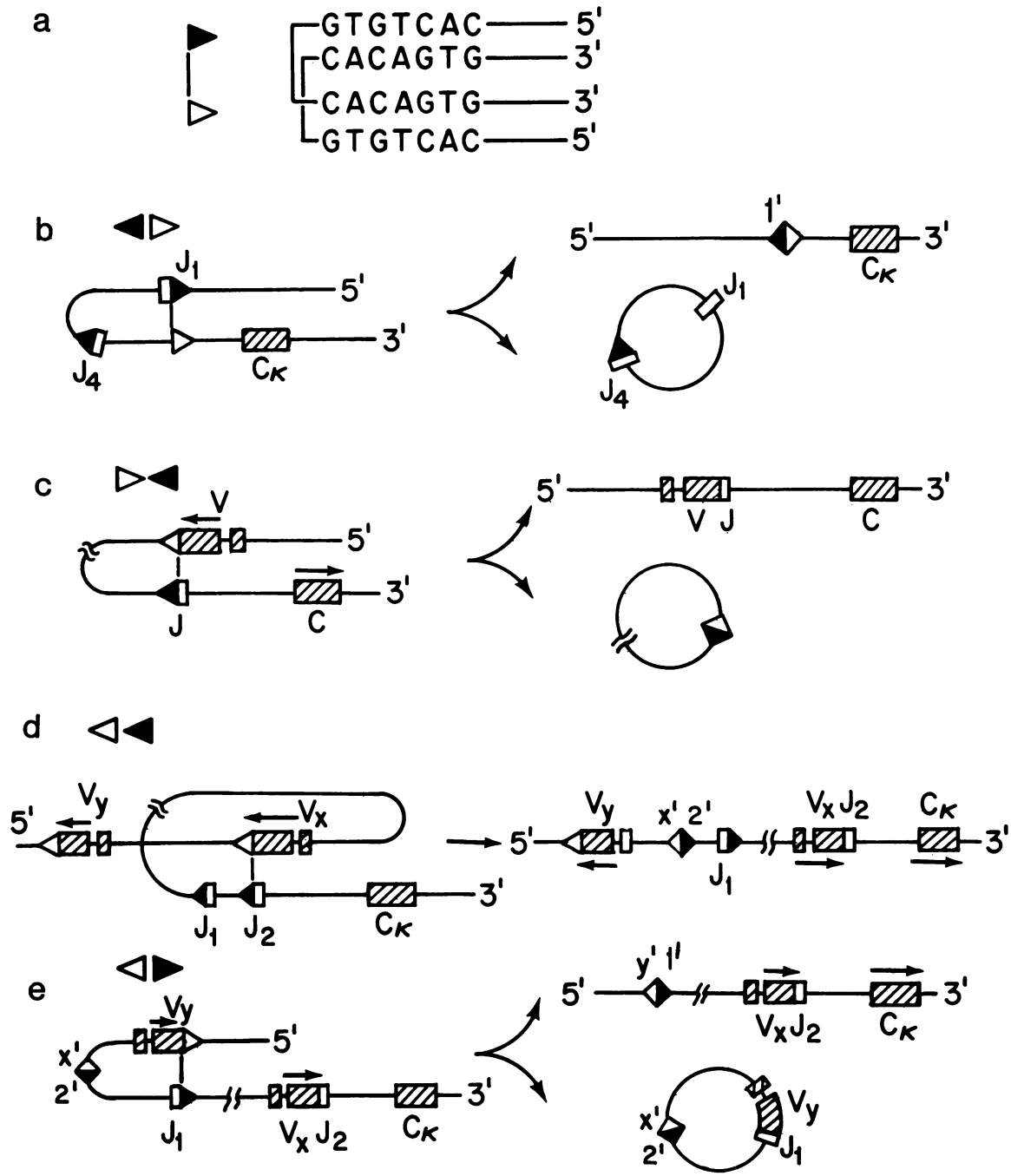


FIG. 13. Model for immunoglobulin gene recombination which relates the fate of recombinase recognition signals to their initial orientation. This model is similar to that previously proposed by Baltimore and his colleagues (1, 29), except that the putative recombinase signals are assumed to have a parallel alignment in the recombination intermediate rather than an antiparallel alignment. As a result the specification of inversion and deletion structures is reversed. (a) Definition of parallel heptamer alignment (▶, J-associated signals; ▷, V-associated signals); (b) generation of deletion present in PCs 8701 and 10916; (c) fusions of V_κ-J_κ, D-J_H, and V_H-D (same transcriptional direction); (d) fusion of V_κ-J_κ (opposite transcriptional direction); (e) loss of a reciprocal joint by a secondary recombination. x' and y', Signals associated with V_x and V_y genes, respectively; 1' and 2', signals associated with the arbitrarily chosen J_1 and J_2 elements, respectively. The orientation of a complete recombination signal is defined as tail-heptamer-spacer-nonamer-head. Thus, V-associated and J-associated heptamers are generally tail-CACAGTG-head and head-CACTGTG-tail, respectively, on the DNA sense strand. The orientation of an isolated heptamer can be specified by its relationship to the consensus sequences of the V-associated and J-associated heptamers. Since the heptamer in the J_κ-C_κ intron conforms to the V consensus, it is drawn as tail-CACAGTG-head.

the κ^+ allele. Interestingly, a similar ratio was observed for the transcriptional activity of the κ^- and κ^+ alleles of PC 8701, indicating that the 2,462-bp deletion which distinguishes the PC 8701 κ^- gene from a normal κ^0 gene does not diminish its transcriptional activity. Thus, the relatively low steady-state level of the 5.5-kb PC 8701 κ^- transcript (Fig. 10) presumably reflects its higher turnover rate compared with that of the normal 8-kb κ^0 transcripts.

For PCs 10916 and 3386, the transcriptional data were in good agreement with the Northern blot analyses of steady-state components. In PC 10916, no transcription products containing κ^0(a) sequences were detected, and the transcriptional activity of the κ^- allele was only a few percent of that of the κ^+ allele. In PC 3386, the rate of transcription of the κ^- allele is three- to fivefold greater than that of the κ^+ allele.

DISCUSSION

Abnormal genes and aberrant recombinations. The six nonproductive κ genes studied here showed a broad spectrum of abnormalities (Fig. 12). One κ^- gene, that in PC 6684, was created by a perfectly normal recombination between a variable-region gene of the $V_\kappa 11$ family and the $J_\kappa 1$ element. However, the gene bears a single point mutation which causes the conventional splice site to be ignored and a cryptic splice site to be utilized, resulting in the inclusion of a premature termination codon in the coding sequence. Whether this mutation resides in the germline $V_\kappa 11$ gene or is generated somatically is unknown. If it was generated somatically, as has been definitively shown for a λ^- allele (21), three possible pathways can be envisioned: (i) it occurred in an unrearranged V gene before VJ recombination, which would be unusual according to the general perception of the mutational mechanism (14, 16, 49; Huppi, Ph.D. thesis); (ii) it occurred concurrently with the recombination event; or (iii) it occurred in a rearranged V gene that was originally part of a κ^+ allele. For this last possibility to be consistent with current models of allelic exclusion, which postulate that recombinational activity ceases when a productive allele is formed (9), it must be assumed that the cessation of recombinational activity is not an irreversible phenomenon.

The other five κ^- genes were involved in aberrant recombinations. One of these, the κ^- gene of PC 2154, retained one extra base pair in a $V_\kappa 5 \rightarrow J_\kappa 3$ recombination, leading to a translational frame shift and premature termination of the encoded κ chain. Although such recombinational imprecision has a negative consequence in the present example, it can, under circumstances in which the reading frame is preserved, serve to increase immunoglobulin diversity (53) or to modify a response to the anti-idiotypic regulatory network (43).

The κ^- allele of PC 3386 also contains a V gene, in this case a member of the $V_\kappa 10$ group. Aberrant recombinational events resulted in two substantial deletions in this κ^- gene: one eliminated the normal VJ junction and the other eliminated a downstream J element and part of the JC intron. This κ^- gene also contains an unusually large number of point mutations immediately downstream of the V gene element. Conceivably, similar upstream mutations may have created the three termination codons that were present in the body of the V gene. We did not discern any homologies or other notable features in the sequences surrounding the deletion sites; therefore, the nature of the recombinational mechanisms that produced these abnormalities remains obscure. Whether the particular $V_\kappa 10$ gene used in this κ^- rearrangement is normal or a pseudogene is not known. However, it is clear that aberrant recombinations contributed to the nonproductivity of the PC 3386 κ^- allele.

In the other three examples of this series, the κ^- alleles involved either a large deletion in the κ^0 locus (PC 8701), a recombination between the κ^0 locus and a DNA segment that did not contain a V gene (PC 7183), or both types of abnormality (PC 10916). The 2,462-bp deletion common to PC 8701 and PC 10916 was produced by the precise fusion of the heptamer, 5′-CACTGTG-3′, that is located on the 5′ flank of $J_\kappa 1$ with an isolated heptamer, 5′-CACAGTG-3′, that is located in the J_κ-C_κ intron 1,100 nucleotides downstream from $J_\kappa 4$ and is identical to those on the 3′ flanks of the V_κ genes. The heptamers, together with 3′-flanking spacer and nonamer sequences, are believed to comprise the recognition signals for a site-specific recombinase that is implicated in the rearrangement of both light- and heavy-chain genes (53). In normal V_κ-J_κ fusion, these signals are always removed from the recombinational joint and are either lost from the genome or displaced to another chromosomal site; in D-J_H and V_H-D recombinations of heavy-chain genes, they are invariably lost from the genome (2, 55). In all of the displaced κ signals characterized to date, the heptamers are precisely fused, just as they are in the PC 8701 and PC 10916 κ^- alleles (19).

A novel feature of the deletion in PCs 8701 and 10916 is that the heptamers were retained at the recombinational joint rather than being lost or displaced to another chromosomal site. This finding can be readily accommodated by a recombination model that posits a relationship between the initial orientation of the recognition signals and their fate (1, 29). In this type of model (Fig. 13), recombination entails an alignment of recognition signals, precise breakage and fusion of heptamer tails, and resealing of the other free ends. The alignment might be either parallel, i.e., complementary with respect to heptamer and nonamer sequences located on the same DNA strand (Fig. 13a), or antiparallel, as conceived by Lewis et al. (29). Antiparallel alignment requires an additional loop in the recombination intermediates. The resultant circular recombination products would lack a centromere and would therefore be unstable and lost from the cells, whereas linear products would be retained. Such a model predicts that interacting heptamers that are initially oriented tail to tail (Fig. 13b) should be retained at the recombination joint, as is the case in the deletion in PCs 8701 and 10916, whereas those with an initial head-to-head orientation (Fig. 13c) should be lost, as in DJ_H joining and V_HD or V_κ-J_κ joining of elements that are in the same transcriptional direction. Moreover, an initial tail-to-head orientation, as would be the case for any V_κ genes that have a transcriptional direction opposite that of C_κ (28), should result in retention of the fused heptamers at the reciprocal joint (Fig. 13d). Secondary recombinations that generate new reciprocal joints and delete the original ones (Fig. 13e) could account for the frequent lack of a reciprocal relationship between the retained fused heptameric structure and the V_κ-J_κ joint (19, 28, 50, 55).

It is important to note that our observation is not inconsistent with the results predicted by other recombination models based on interchromatid exchange (19, 55) or episomal reinsertion (50). However, a particularly attractive feature of the intrachromatid recombination model described above is the manner in which the initial orientation of the recombination signals unequivocally predicts the fate of the recombination products. In the two cases for which the initial orientation is known with certainty, D-J_H fusion and the deletion in PCs 8701 and 10916, this prediction is confirmed. By the same token, knowledge of the recombination product and the orientation of one set of signals should allow prediction of the orientation of the other set. For example, there are recombinations which result in the deletion of the region containing the C_κ exon and which presumably involve the same heptamer signal as that used in the deletion in PCs 8701 and 10916 (11). Since fused heptamers were not found at the borders of these deletions, we

would predict that the recombinational events that produce C_κ deletions utilize a recombination signal that is located 3′ of the C_κ region and oriented in the same direction as the signals in the conventional J_κ elements.

In both the PC 7183 and PC 10916 κ^- alleles, the κ locus recombined with DNA that did not contain V gene elements. These κ^- rearrangements had some similarities, although the details of the recombinational events were quite different. In PC 7183, the foreign DNA, a single-copy DNA segment from chromosome 15 joined to a ca. 700-nucleotide segment of the μ switch region, was rearranged to a site 1,321 nucleotides downstream of $J_\kappa 4$, presumably by two independent events: transposition of the μ switch sequence to chromosome 15 and then a reciprocal translocation between this region of chromosome 15 and the region of chromosome 6 bearing the κ locus (51, 56). In PC 10916, the foreign DNA, a single-copy sequence of unknown chromosomal location, was rearranged to a site 887 nucleotides upstream of $J_\kappa 1$. Conceivably, this DNA may also have been brought to the κ locus by translocation. Both of these PCs contain a rearranged copy of the far-upstream region of the κ locus (the region containing the pseudopromoter) elsewhere in their genomes. In PC 7183, this region was shown to reside on the reciprocal of the chromosome 15-chromosome 6 translocation that generated the κ^- allele. Whether this was also the case in PC 10916 remains to be established. In neither of these κ^- alleles is there any evidence of sequences that might be involved in homologous pairing. Moreover, although similar to translocations between the *myc* oncogene and other nonproductive immunoglobulin alleles (38), neither of these κ^- rearrangements appears to involve the *myc* locus (18, 56).

Consequences of κ^- lesions for transcription, processing, and turnover. All of the V gene-containing κ^- alleles in this study (PCs 2154, 3386, and 6684) are actively transcribed. Indeed, the nuclear content of κ^- primary transcripts was found to be comparable to or even greater than that of κ^+ primary transcripts. In PC 3386, the greater abundance of κ^- transcripts is apparently related to a higher rate of transcription of the κ^- allele than of the κ^+ allele. The reason for the greater transcriptional activity of the κ^- allele is presently unclear. It might be a consequence of its abnormal structure, or it could reflect some intrinsic differences between the $V_\kappa 10$ and $V_\kappa 21$ families. Owing to a loss (PC 3386) or an alteration (PC6684) of a 5′ splice junction, the κ^- transcripts of PC 3386 and PC 6684 are incapable of being properly spliced. In both cases the transcripts are processed into defective mRNAs by the recognition of alternative (cryptic) 5′ splice sites. The use of cryptic splice sites when normal ones are mutated is apparently a common characteristic of RNA splicing mechanisms (54, 49). In some cases the processing efficiency might be reduced when cryptic sites are used, and this could also contribute to the relatively high abundance of κ^- primary transcripts.

The data presented here as well as in our earlier studies (57) indicate that in PCs the transcriptional activity of a κ^+ allele (or a V gene-containing κ^- allele) is generally about four- to fivefold higher than that of the κ^0 allele. Since the enhancer element in the J_κ-C_κ intron is common to both κ^+ and κ^0 alleles, it might be supposed that the higher transcriptional activity of κ^+ alleles reflects either a superiority of V_κ gene promoters over the pseudopromoter associated with the κ^0 allele (57) or the greater proximity of the promoter and enhancer elements in κ^+ alleles (3 to 4 kb) than in κ^0 alleles (ca. 6.5 kb). The latter possibility seems unlikely, however, when we consider the transcriptional data from PC 8701. The 2.5-kb deletion in the κ^- allele of this PC reduced the separation of pseudopromoter and enhancer to about 4 kb, yet its transcriptional activity was essentially the same as that of a normal κ^0 allele. Thus, we favor the explanation based on promoter strength superiority. In this regard it is worth noting that the highly conserved octanucleotide that appears to be an essential part of the V_κ promoter is ATTTGCAT (13, 37), whereas its presumptive counterpart in the κ^0 pseudopromoter is ATGTAAAT, a close homolog of the inverted octanucleotide common to V_H gene promoters (13, 37). Furthermore, in the pseudopromoter the octanucleotide is located closer to the transcriptional start site and TATA box than it is in the V_κ promoter (13, 37, 57). Conceivably the spacing and orientation of these sequences could be important for optimization of transcriptional activity.

The relative contributions of promoter and enhancer elements in determining transcriptional activity are dramatically illustrated by the κ^- alleles of PCs 10916 and 7183. In these two PCs a chromosomal region that is normally transcriptionally inactive recombined into the κ locus and presumably fell under the influence of the κ enhancer. In both PCs, transcripts were produced from the foreign DNA, but at levels far below those of κ^+, κ^0, and V gene-containing κ^- alleles. The rate of transcription of the κ^- allele in PC 10916 was only about 3% of that of the κ^+ allele, and the accumulation of poly(A)$^+$ κ^- transcripts in PC 10916 and PC 7183 nuclei is only 2 to 10% of that of the κ^+ primary transcripts. The κ^- components of PC 10916 extend into the κ locus and may have been transcribed in the same direction as normal κ transcripts. The κ^- component of PC 7183 does not extend into the κ locus and may have been transcribed in the opposite direction, although additional data are needed to establish this point definitively. In any case, the promoters that generated these transcripts seem to be considerably less effective in PCs than the V_κ promoters and κ^0 pseudopromoter.

In an earlier study (31), the DNA of these transcriptionally depressed (previously thought to be transcriptionally inactive) κ^- alleles was found to be hypomethylated to the same extent as the κ^+ and κ^0 alleles. Our observations are thus consistent with a hierarchical relationship in which a transcriptionally competent chromatin structure is established in the region encompassing the κ locus by the interaction of the enhancer element and *trans*-acting factors unique to cells of the B-lymphoid lineage (12, 30, 46, 52). Any promoter element within the domain of influence of this enhancer would presumably be active in PCs, although the extent of activity can obviously vary by orders of magnitude depending on the particular characteristics of the promoter sequences and on other features of chromatin structure (15, 46).

A final point concerns the influence of transcript structure on turnover. Although deletion of the entire J_κ region in the κ^- allele of PC 8701 has no detectable effect on its transcription rate, it engenders a 5- to 10-fold reduction in the nuclear accumulation of the 5.5-kb primary transcript compared with that of 8-kb transcripts from normal κ^0 alleles. Thus, it would seem that the deletion causes a marked acceleration in the intranuclear turnover rate. This acceleration might be related to an observed difference in breakdown pathway and ultimately to a critical difference in RNA conformation.

ACKNOWLEDGMENTS

We thank Martin Weigert for stimulating discussion and critical comments, Beatrice Conner and Dorothy Feszko for excellent technical assistance, and Joni Brill and Roy Riblet for supplying us with

samples of PC and liver DNA. A.C.P. was on leave of absence from the Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland.

This research was supported by a grant to R.P.P. from the National Science Foundation and grants to R.P.P. and B.V.N. from the National Institutes of Health. An appropriation from the Commonwealth of Pennsylvania is also acknowledged.

LITERATURE CITED

1. **Alt, F., and D. Baltimore.** 1982. Joining of immunoglobulin heavy chain gene segments: implications from a chromosome with evidence of three D-J_H fusions. Proc. Natl. Acad. Sci. U.S.A. **79**:4118–4122.
2. **Alt, F. W., G. D. Yancopoulos, T. K. Blackwell, C. Wood, E. Thomas, M. Boss, R. Coffman, N. Rosenberg, S. Tonegawa, and D. Baltimore.** 1984. Ordered rearrangement of immunoglobulin heavy chain variable region segments. EMBO J. **3**:1209–1219.
3. **Altenburger, W., M. Steinmetz, and H. G. Zachau.** 1980. Functional and nonfunctional joining in immunoglobulin light chain genes of a mouse myeloma. Nature (London) **287**:603–607.
4. **Bentley, D. L., and T. H. Rabbitts.** 1980. Human immunoglobulin variable region genes—DNA sequences of two V_κ genes and a pseudogene. Nature (London) **288**:730–733.
5. **Benton, W. D., and R. W. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques in situ. Science **196**:180–182.
6. **Bergman, Y., D. Rice, R. Grosschedl, and D. Baltimore.** 1984. Two regulatory elements for immunoglobulin κ light chain gene expression. Proc. Natl. Acad. Sci. U.S.A. **81**:7041–7045.
7. **Bernard, O., N. M. Gough, and J. M. Adams.** 1981. Plasmacytomas with more than one immunoglobulin κ mRNA: implications for allelic exclusion. Proc. Natl. Acad. Sci. U.S.A. **78**:5812–5816.
8. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
9. **Coleclough, C.** 1983. Chance, necessity and antibody gene dynamics. Nature (London) **303**:23–26.
10. **Coleclough, C., R. P. Perry, K. Karjalainen, and M. Weigert.** 1981. Aberrant rearrangements contribute significantly to the allelic exclusion of immunoglobulin gene expression. Nature (London) **290**:372–377.
11. **Durdik, J., M. W. Moore, and E. Selsing.** 1984. Novel κ light-chain gene rearrangements in mouse λ light chain-producing B lymphocytes. Nature (London) **307**:749–752.
12. **Ephrussi, A., G. M. Church, S. Tonegawa, and W. Gilbert.** 1985. B lineage-specific interactions of an immunoglobulin enhancer with cellular factors *in vivo*. Science **227**:134–140.
13. **Falkner, F. G., and H. G. Zachau.** 1984. Correct transcription of an immunoglobulin κ gene requires an upstream fragment containing conserved sequence elements. Nature (London) **310**: 71–84.
14. **Gearhart, P. J., N. D. Johnson, R. Douglas, and L. Hood.** 1981. IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. Nature (London) **291**:29–34.
15. **Gerondakis, S., A. Boyd, O. Bernard, E. Webb, and J. M. Adams.** 1984. Activation of immunoglobulin μ gene expression involves stepwise demethylation. EMBO J. **3**:3013–3021.
16. **Gorski, J., P. Rollini, and B. Mach.** 1983. Somatic mutations of immunoglobulin variable genes are restricted to the rearranged V gene. Science **220**:1179–1181.
17. **Groudine, M., M. Peretz, and H. Weintraub.** 1981. Transcriptional regulation of hemoglobin switching in chicken embryos. Mol. Cell. Biol. **1**:281–288.
18. **Harris, L. J., R. B. Lang, and K. B. Marcu.** 1982. Non-immunoglobulin-associated DNA rearrangements in mouse plasmacytomas. Proc. Natl. Acad. Sci. U.S.A. **79**:4175–4179.
19. **Hochtl, J., C. R. Müller, and H. G. Zachau.** 1982. Recombined flanks of the variable and joining segments of immunoglobulin genes. Proc. Natl. Acad. Sci. U.S.A. **79**:1383–1387.
20. **Hohn, B.** 1979. *In vitro* packaging of λ and cosmid DNA. Methods Enzymol. **68**:299–309.
21. **Hozumi, N., G. E. Wu, H. Murialdo, L. Roberts, D. Vetter, W. L. Fife, M. Whiteley, and P. Sadowski.** 1981. RNA splicing mutation in an aberrantly rearranged immunoglobulin λ1 gene. Proc. Natl. Acad. Sci. U.S.A. **78**:7019–7023.
22. **Huang, H., S. Crews, and L. Hood.** 1981. An immunoglobulin V_H pseudogene. J. Mol. Appl. Genet. **1**:93–101.
23. **Kabat, E. A., T. T. Wu, H. Bilofsky, M. Reid-Miller, and H. Perry.** 1983. Sequences of proteins of immunological interest, p. 45–57. U.S. Department of Health and Human Services, Washington, D.C.
24. **Karn, J., S. Brenner, L. Barnett, and G. Cesareni.** 1980. Novel bacteriophage λ cloning vector. Proc. Natl. Acad. Sci. U.S.A. **77**:5172–5176.
25. **Kelley, D. E., C. Coleclough, and R. P. Perry.** 1982. Functional significance and evolutionary development of the 5′-terminal regions of immunoglobulin variable-region genes. Cell **29**:681–689.
26. **Kwan, S.-P., E. E. Max, J. G. Seidman, P. Leder, and M. D. Scharff.** 1981. Two kappa immunoglobulin genes are expressed in the myeloma S107. Cell **26**:57–66.
27. **Leder, P., D. Tiemeier, and L. Enquist.** 1977. EK2 derivatives of bacteriophage lambda useful in the cloning of DNA from higher organisms: the λgtWES system. Science **196**:175–177.
28. **Lewis, S., A. Gifford, and D. Baltimore.** 1984. Joining of V_κ to J_κ gene segments in a retroviral vector introduced into lymphoid cells. Nature (London) **308**:425–428.
29. **Lewis, S., N. Rosenberg, F. Alt, and D. Baltimore.** 1982. Continuing kappa-gene rearrangement in a cell line transformed by Abelson murine leukemia virus. Cell **30**:807–816.
30. **Mather, E. L., and R. P. Perry.** 1981. Transcriptional regulation of immunoglobulin genes. Nucleic Acids Res. **9**:6855–6867.
31. **Mather, E. L., and R. P. Perry.** 1983. Transcriptional regulation of κ immunoglobulin genes. Proc. Natl. Acad. Sci. U.S.A. **80**:4689–4693.
32. **Max, E. E., J. V. Maizel, and P. Leder.** 1981. The nucleotide sequence of a 5.5 kb DNA segment encoding the mouse kappa immunoglobulin J and C genes. J. Biol. Chem. **256**:5116–5120.
33. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
34. **McKnight, G. S., and R. Palmiter.** 1979. Transcriptional regulation of the ovalbumin and conalbumin genes by steroid hormones in chick oviduct. J. Biol. Chem. **254**:9050–9058.
35. **Nelson, K. J., J. Haimovich, and R. P. Perry.** 1983. Characterization of productive and sterile transcripts from the immunoglobulin heavy-chain locus: processing of μ_m and μ_s mRNA. Mol. Cell. Biol. **3**:1317–1332.
36. **Neumaier, P. S., and H. G. Zachau.** 1983. Nucleotide sequence of a region downstream of the mouse C_κ immunoglobulin gene. Nucleic Acids Res. **11**:3631–3636.
37. **Parslow, T., D. L. Blair, W. J. Murphy, and D. K. Granner.** 1984. Structure of the 5′ ends of immunoglobulin genes: a novel conserved sequence. Proc. Natl. Acad. Sci. U.S.A. **81**: 2650–2654.
38. **Perry, R. P.** 1983. Consequences of *myc* invasion of immunoglobulin loci: facts and speculation. Cell **33**:647–649.
39. **Perry, R. P.** 1984. What controls the transcription of immunoglobulin genes? Nature (London) **310**:14–15.
40. **Perry, R. P., C. Coleclough, and M. Weigert.** 1981. Reorganization and expression of immunoglobulin genes: status of allelic elements. Cold Spring Harbor Symp. Quant. Biol. **45**:925–933.
41. **Perry, R. P., D. E. Kelley, C. Coleclough, J. G. Seidman, P. Leder, S. Tonegawa, G. Matthyssens, and M. Weigert.** 1980. Transcription of mouse κ-chain genes: implications for allelic exclusion. Proc. Natl. Acad. Sci. U.S.A. **77**:1937–1941.
42. **Picard, D., and W. Schaffner.** 1984. A lymphocyte-specific enhancer in the mouse immunoglobulin kappa gene. Nature (London) **307**:80–82.
43. **Pollok, B. A., M. Vakil, J. F. Kearney, and R. P. Perry.** 1984. A biological consequence of variation in the site of D-J_H gene rearrangement. Nature (London) **311**:376–379.
44. **Queen, C., and J. Stafford.** 1984. Fine mapping of an im-

munoglobulin gene activator. Mol. Cell. Biol. **4**:1042–1049.
45. **Rimm, D. L., D. Horness, J. Kucera, and F. R. Blattner.** 1980. Construction of coliphage lambda Charon vectors with Bam HI cloning sites. Gene **12**:301–309.
46. **Rose, S. M., and W. T. Garrard.** 1984. Differentiation-dependent chromatin alterations precede and accompany transcription of immunoglobulin light chain genes. J. Biol. Chem. **259**:8534–8544.
47. **Schibler, U., O. Hagenbüchle, P. K. Wellauer, and A. C. Pittet.** 1983. Two promoters of different strengths control the transcription of the mouse alpha-amylase gene amy-1[a] in the parotid gland and the liver. Cell **33**:501–508.
48. **Seidman, J. G., and P. Leder.** 1980. A mutant immunoglobulin light chain is formed by aberrant DNA- and RNA-splicing events. Nature (London) **286**:779–783.
49. **Selsing, E., and U. Storb.** 1981. Somatic mutation of immunoglobulin light-chain variable-region genes. Cell **25**:47–58.
50. **Selsing, E., J. Voss, and U. Storb.** 1984. Immunoglobulin gene 'remnant' DNA—implications for antibody gene recombination. Nucleic Acids Res. **12**:4229–4246.
51. **Shapiro, M., and M. Weigert.** 1984. Aberrant rearrangements at the murine light chain locus in plasmacytomas. Curr. Top. Microbiol. Immunol. **113**:190–191.
52. **Storb, U., R. L. O'Brien, M. D. McMullen, K. A. Gollahon, and R. L. Brinster.** 1984. High expression of cloned immunoglobulin κ gene in transgenic mice is restricted to B lymphocytes. Nature (London) **310**:238–241.
53. **Tonegawa, S.** 1983. Somatic generation of antibody diversity. Nature (London) **302**:575–581.
54. **Treisman, R., S. H. Orkin, and T. Maniatis.** 1983. Specific transcription and RNA splicing defects in five cloned β-thalassaemia genes. Nature (London) **302**:591–596.
55. **Van Ness, B. G., C. Coleclough, R. P. Perry, and M. Weigert.** 1982. DNA between V and J gene segments of immunoglobulin κ light chain is frequently retained in cells which rearrange the κ locus. Proc. Natl. Acad. Sci. U.S.A. **79**:262–266.
56. **Van Ness, B. G., M. Shapiro, D. E. Kelley, R. P. Perry, M. Weigert, P. D'Eustachio, and F. Ruddle.** 1983. Aberrant rearrangement of the κ light-chain locus involving the heavy-chain locus and chromosome 15 in a mouse plasmacytoma. Nature (London) **301**:425–427.
57. **Van Ness, B. G., M. Weigert, C. Coleclough, E. L. Mather, D. E. Kelley, and R. P. Perry.** 1981. Transcription of the unrearranged mouse C_κ locus: sequence of the initiation region and comparison of activity with a rearranged V_κ-C_κ gene. Cell **27**:593–602.
58. **Walfield, A., E. Selsing, B. Arp, and U. Storb.** 1981. Misalignment of U and J gene segments resulting in a nonfunctional immunoglobulin gene. Nucleic Acids Res. **9**:1101–1109.
59. **Wieringa, B., F. Meyer, J. Reiser, and C. Weissmann.** 1983. Unusual sequence of cryptic splice sites utilized in the β-globin gene following inactivation of an authentic 5′ splice site by site-directed mutagenesis. Nature (London) **301**:38–43.