Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 5709–5713, September 1983
Immunology

Rabbit immunoglobulin κ genes: Structure of a germline b4 allotype J–C locus and evidence for several b4-related sequences in the rabbit genome

(joining region/nucleotide sequence/latent allotype)

LAURENT EMORINE, KEVIN DREHER*, THOMAS J. KINDT, AND EDWARD E. MAX†

Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

Communicated by Philip Leder, June 6, 1983

ABSTRACT **To investigate the genetic mechanism by which certain rabbits can express immunoglobulins unexpected on the basis of their pedigree (i.e., "latent" allotypes), we have begun a study of the rabbit immunoglobulin κ gene locus. Here we report the structure of a germline genomic clone that encodes the b4 allotype of rabbit κ immunoglobulin and corresponds to the κ gene expressed by the rabbit–mouse hybridoma 12F2. The nucleotide sequences of the joining (J) and constant (C) regions reveal structures generally similar to the homologous mouse and human loci, although only one of the five J-like sequences of this rabbit gene is apparently expressed. Southern blotting analysis of DNA from several rabbit allotypic strains by using probes derived from the cloned b4 gene demonstrates that, in contrast to mouse and human, rabbits possess multiple κ-related sequences. Rabbits of the nominal b4, b5, b6, and b9 allotypes each contain at least two b4-related sequences that are associated with their own J regions and that are highly homologous to the cloned b4 gene in both coding and flanking regions.**

κ light chains of the domestic rabbit are classified on the basis of serological and structural features of their constant (C) regions into the allotypes known as b4, $b4^v$, b5, b6, and b9; an additional κ-like isotype known as "κ2" has also been described in some rabbits. Early serological studies during breeding experiments revealed that expression of the b series allotypes appeared to be governed by inheritance of allelic structural genes. Subsequently, sensitive and specific allotype assays showed that the serum from some rabbits contained, in addition to the highly expressed "nominal" allotypes, low levels of immunoglobulins bearing allotypic specificities unexpected from the rabbits' pedigree (1–3). On the other hand, there is no evidence that every rabbit is capable of expressing latent allotypes or that any rabbit can express more than one latent allotype of the b series. [For an extensive and critical discussion of latent allotypes refer to Kindt and Yarmush (4).] The fact that a rabbit can express an allotype unanticipated from its genetic background suggests that at least some rabbits harbor additional κ genes besides the nominal genes usually expressed. What inherited mechanism might prevent the expression of such latent structural genes except at low levels in occasional animals?

We have begun an investigation of this question by cloning a genomic rabbit κ C region gene of the b4 allotype. Nucleotide sequence analysis of the gene reveals an apparently functional C domain and five joining (J)-like sequences, of which only one appears to be expressed. Southern blots probed with fragments from the cloned gene indicate that: (*i*) the cloned C_κ gene rearranges in a b4-secreting rabbit–mouse hybridoma and is thus a functional b4 gene; (*ii*) a short C-region coding sequence probe from the gene identifies approximately 10 related sequences in rabbits of all allotypes [in confirmation of earlier work by Heidmann and Rougeon (5)]; and (*iii*) among these related sequences, probes that include DNA flanking the C gene identify in each allotype two sequences—designated "b4A" and "b4B"—which are strongly homologous to the b4 gene. These include several bands that are unlikely to represent the expressed nominal genes of the respective rabbits and that could represent "latent" structural genes, although our present data cannot establish this.

MATERIALS AND METHODS

Isolation of Sperm DNA. Crude rabbit ejaculates were washed three times with proteinase K buffer (50 mM Tris·HCl, pH 8.3/10 mM EDTA/10 mM NaCl). The suspension was then made 0.1% $NaDodSO_4$/1% dithiothreitol and incubated for 30 min at 50°C. Proteinase K was added to 1 mg/ml and digestion was carried out at 50°C until clarification (1–3 hr) (6). The DNA solution was then extracted as described by Blin and Stafford (7).

Library Construction and Clone Characterization. A partial *Mbo* I library of b4 genomic DNA was constructed in Charon 28 (8) DNA by using the packaging procedure of Enquist and Sternberg (9) and the lysogenic strains BHB2688 and BHB2690 (10). Two million plaques from the amplified library (11) were screened by using a nick-translated probe derived from a cloned rabbit C_κ cDNA, pB4D5 (12) (see map in Fig. 3). Seventeen hybridizing clones were purified. From restriction analysis of DNA minipreps (13), they all apparently contained fragments derived from the same chromosomal region.

Southern Blotting Analysis. Southern blots (14) of genomic DNA were hybridized in the presence of 10% dextran sulfate and 40% formamide (15) and washed with maximal stringency in 15 mM sodium chloride/1.5 mM sodium citrate, pH 7/0.05% $NaDodSO_4$ at 45°C.

Sequence Determination and Computer Analysis. All sequence determinations were done on single-stranded DNA derived from fragments subcloned in the M13 mp8 and mp9 phages (16). The sequence was determined by the Sanger dideoxynucleotide terminators methods (17) by using the BRL M13 sequence analysis kit. The Korn–Queen program (18) was used for sequence analysis.

RESULTS

Isolation and Characterization of a Germline J_κ–C_κ Locus from a b4 Homozygous Rabbit. An amplified genomic library

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: C, constant; J, joining; V, variable; kb, kilobase(s); bp, base pair(s).

* Present address: Dept. of Biochemistry, School of Medicine, Medical Center, West Virginia Univ., Morgantown, WV 26506.
† To whom correspondence and reprint requests should be addressed.

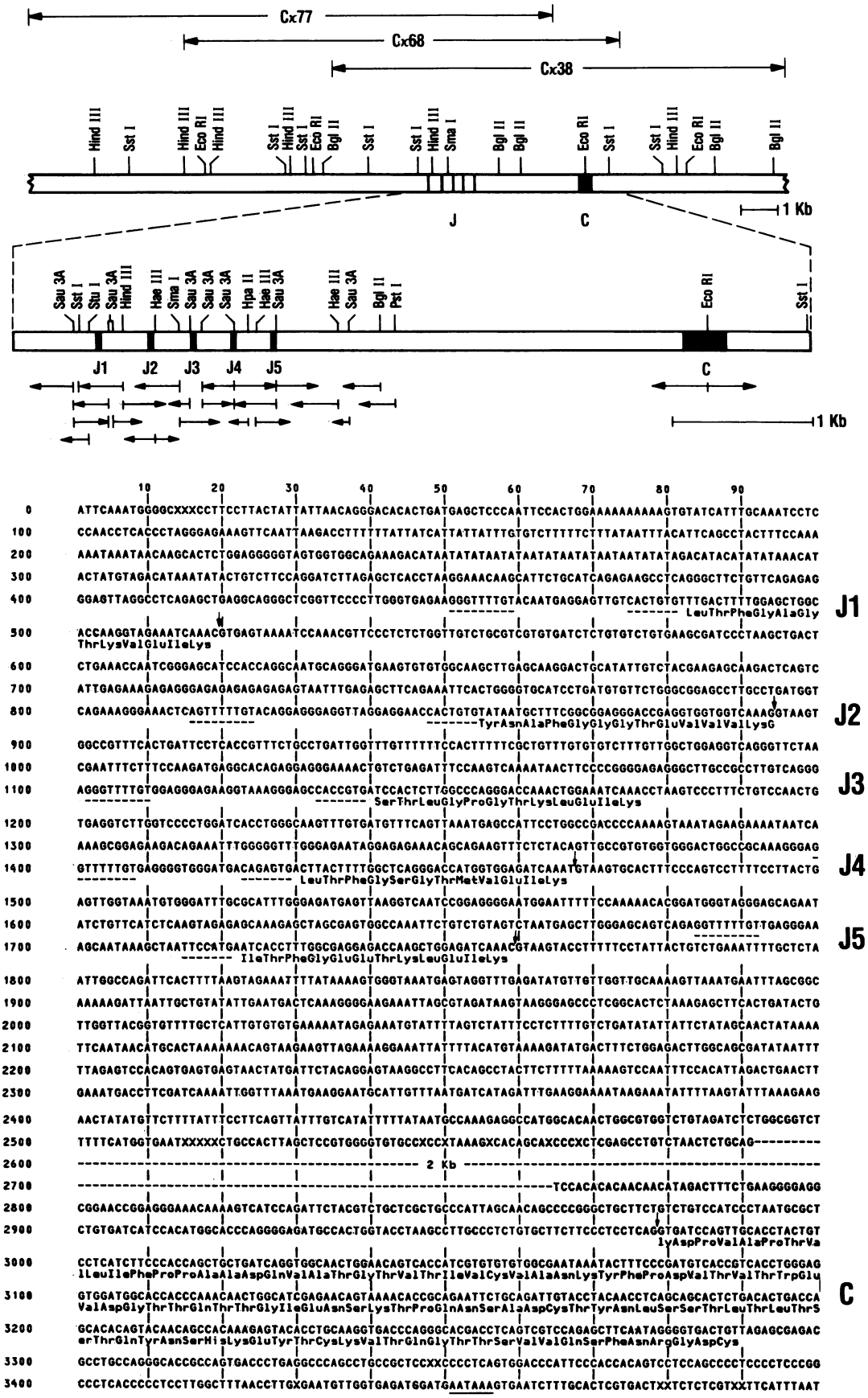

FIG. 1. Structural characterization of a rabbit κ Ig J and C locus. From the three overlapping clones indicated at the top ($C_κ77$, $C_κ68$, and $C_κ38$) the J and C regions were examined by nucleotide sequence analysis. Each M13 subclone analyzed is represented under the map by arrows indicating the origin, direction, and extent of sequence determination. The sites of nonamer and heptamer recombination signals 5′ to the J coding region are underlined for each J gene, and functional mRNA splice sites are indicated by vertical arrows.

```
J1    TGGGTGAGAAGGGTTTTGTACAATGAGGAGTTGTCACTGTGT  TTGACTTTTGGAGCTGGCACCAAGGTAGAAATCAAACGTGAGTA
            --*------  (15 bp)     -------   LeuThrPheGlyAlaGlyThrLysValGluIleLys

J2    TCAGTTTTTGTACAGGAGGGAGGTTAGGAGGAACCACTGTGTATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAAGGTAAGTG
      *--------          (23 bp)     -------TyrAsnAlaPheGlyGlyGlyThrGluValValValLys

J3    GGAGGGTTTTGTGGAGGGAGAAGGTAAAGGGAGCCACCGTGA  TCCACTCTTGGCCCAGGGACCAAACTGGAAATCAAACCTAAGTC
         --*------       (22 bp)     ---*---   SerThrLeuGlyProGlyThrLysLeuGluIleLys *

J4    CCGCAAAGGGAGGTTTTTGTGAGGGGTGGGATGACAGAGTGA  CTTACTTTTGGCTCAGGGACCATGGTGGAGATCAAATGTAAGTG
                  ---------  (14 bp)     --**---   LeuThrPheGlySerGlyThrMetValGluIleLys

J5    AGAGGTTTTTGTTGAGGGAAAGCAATAAAGCTAATTCCATGA  ATCACCTTTGGCGAGGAGACCAAGCTGGAGATCAAACGTAAGTA
         ---------       (22 bp)     **-**--   IleThrPheGlyGluGluThrLysLeuGluIleLys

b4 cDNA pB4D5                          AGTAGTAATGTTGAGAATGTTTTCGGCGGAGGGACCGAGGTGGTGGTCAAAGGTGATCC
                                       SerSerAsnValGluAsnValPheGlyGlyGlyThrGluValValValLysGlyAspPr

                                                                98                  108
b4 K LIGHT CHAIN J REGION               ---------hv3--------PheGlyGlyGlyThrGluValValValLysGlyAspPr
```

FIG. 2. Comparison of the germline J_κ sequences with expressed b4 mRNA and protein J regions. The five J sequences found in our germline b4 κ clone are presented, aligned to highlight homologies. The nonamer and heptamer elements are underlined and the spacing between them is indicated; arrows indicate mRNA donor splice sites. Deviations from the consensus sequences for these features are indicated by asterisks. Below the germline sequences is the sequence of the corresponding region of the cDNA clone pB4D5 (12). Except for a Gly/Thr substitution at position 100 in a single protein, all 25 available b4 κ amino acid sequences (22) have the same sequence between residues 98 and 108 (bottom line), which matches the sequence encoded by the germline J2. Thus, J2 is the only germline J sequence known to be expressed.

of sperm DNA from a homozygous b4 rabbit was screened with a b4 C_κ probe. Three different overlapping genomic clones were obtained, allowing the characterization of a 22-kilobase (kb) portion of chromosomal DNA containing a J_κ–C_κ locus (Fig. 1).

Nucleotide sequence analysis by the M13-dideoxy method (16) revealed a single C region sequence and a cluster of five J regions (Fig. 1). The C region sequence precisely matches the structure of the two b4 cDNA clones previously described (12, 19) and yields an amino acid translation that agrees with the two published protein sequences of b4 chains (20, 21) except for the replacement of asparagine at position 167 by aspartic acid in the Chen *et al.* (20) sequence. This replacement is probably a sequencing error, but an alternative form of b4 κ chain cannot be ruled out.

All five J sequences translate to amino acid sequences homologous to human or mouse J regions, and all are flanked by sequences homologous to the nonamer and heptamer elements thought to be involved in variable (V)–J recombination. In addition, the spacing of these J region sequences, roughly 0.3 kb apart, is similar to that seen in the homologous loci of mouse and human. However, the cDNA-derived J probe hybridized only to its germline counterpart, designated J2. Furthermore, comparison of the five germline J regions to known b4 κ light chain amino acid sequences indicates that only J2 can account for the observed sequences (Fig. 2).

The Cloned b4 κ Gene Is a Nominal b4 κ Gene. Genomic blots of rabbit DNA hybridized with a rabbit κ C region probe derived from a cDNA clone have indicated that rabbits contain more than one sequence homologous to such a probe (5, 12). It was therefore important to establish whether our cloned genomic gene represents an expressible κ sequence and to determine its relationship to the several bands observable on Southern blots, bands which may include unexpressed homologous sequences or pseudogenes. To this end, digests of b4 germline genomic DNA and DNA from the cloned gene were run in parallel on the same gel, blotted, and hybridized with a b4 κ probe; hybridizing bands from the cloned gene were found to comigrate exactly with strong bands observable in corresponding digests of genomic b4 DNA (data not shown). To determine whether this gene rearranges in b4-expressing cells, digests of germline b4 DNA and DNA from the b4-expressing hybridoma 12F2 (23) were compared by Southern blot analysis (Fig. 3). In Fig. 3, the sizes of the bands corresponding to the cloned gene are enclosed in squares. The map in Fig. 1 indicates which of these bands would be shifted if a V region gene recombined with J2 (i.e., those bands representing fragments that span the J2 sequence: the 7.8-kb *Eco*RI band, the 4.5-kb *Sst* I + *Eco*RI band, the 5.4-kb *Sst* I band, and 7.0-kb *Hin*dIII band), and indeed all are shifted in the hybridoma DNA. In contrast, the 5.8-kb *Bgl* II band (which ends between the J segments and the C region) and the 3.0-kb *Eco*RI band (which extends 3′ of the C region) remain unchanged in the hybridoma DNA as expected, because they represent only sequences downstream of the recombination site at J2. The findings that—(*i*) the germline bands that shift in the hybridoma DNA all correspond to fragments in our cloned gene and (*ii*) the sequence

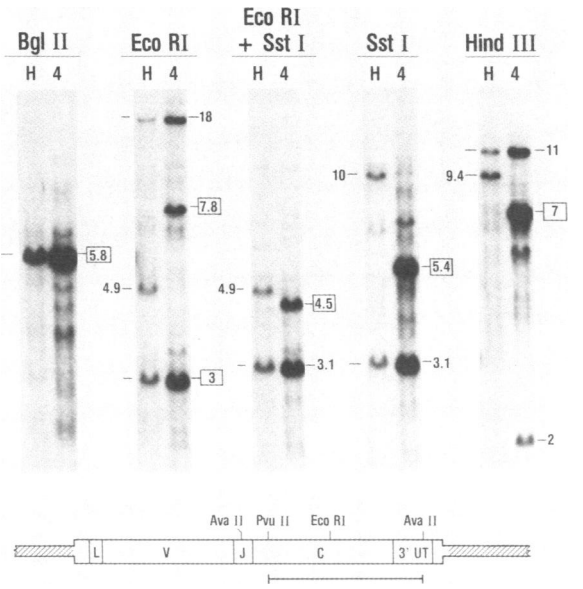

FIG. 3. A rabbit–mouse hybridoma that secretes a rabbit b4 κ chain shows rearrangement of the genomic b4 bands corresponding to the cloned locus. The 12F2 hybridoma DNA ("H") and b4 DNA ("4," isolated from a b4 homozygous rabbit liver) were digested with the enzymes indicated and electrophoresed in a 0.7% agarose gel. Nitrocellulose blots (14) were hybridized to a C region probe (*Pvu* II to *Ava* II) isolated from the pB4D5 cDNA clone, as shown in the map below. The numbers on the left side of a double lane indicate the size (in kb) of the fragments specific to 12F2, which represent the rearrangement of the expressed gene. Bands present in germline b4 or in both DNAs are indicated by numbers on the right hand side and by dashes on the left side. The boxed numbers correspond to fragment sizes observed in the cloned b4 locus. None of the intense bands visible in the hybridoma DNA is due to the mouse contribution to the hybridoma genome because parallel lanes of DNA from the murine fusion partner PU showed only very faint bands.

of this clone exactly matches the cDNA sequence—both indicate that this clone represents the germline form of the b4 κ gene expressed in the hybridoma. Because the hybridoma was derived from the spleen of a homozygous b4 rabbit, the expressed κ chain is a nominal b4 protein. We therefore call our gene b4Nb4 to indicate that it encodes a b4 sequence and is the nominal gene derived from a b4-expressing rabbit.

Southern Blot Analysis of b4-Related Sequences in Rabbit Genomic DNA. In an effort to characterize the b4-related sequences present in rabbit DNA, we compared Southern blots of genomic DNA from homozygous b4^v, b4, b5, b6, and b9 rabbits (Fig. 4). Because these rabbits are not inbred, in interpreting the blots the possibility must be kept in mind that the band patterns we have observed for DNA of a given allotype may not be representative of all rabbits of that allotype.

The 342-base pair (bp) *Pvu* II–*Ava* II "coding" probe, which includes coding sequence and 71 bases of 3′ untranslated region, detects a complex pattern of bands in *Eco*RI-digested DNA from all five allotypes (left panel of Fig. 4). However, because an *Eco*RI site is present within the b4 C region coding sequence (position 3,151 in Fig. 1) and may be retained in b4-related sequences, a single C-region sequence may give rise to two *Eco*RI fragments detected by the probe. Amino acid sequences of different allotype κ chains at the position corresponding to the *Eco*RI site in the b4 coding region [the Gln-Asn-Ser at residues 166–168 in Kabat (22) numbering system] indicate that the nominal

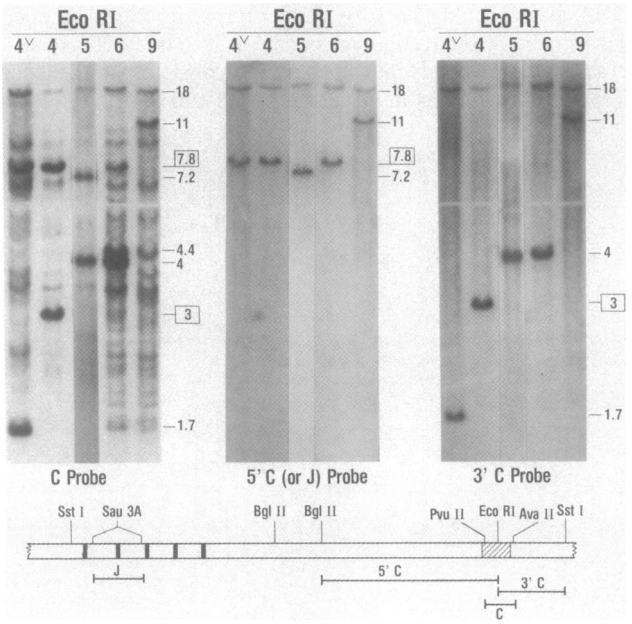

FIG. 4. b4-related genomic sequences in homozygous allotype rabbits demonstrate structural characteristics unanticipated for their nominal allotype structural gene. Southern blots of sperm DNAs from homozygous b4^v, b4, b5, b6, and b9 rabbits (4^v, 4, 5, 6, and 9, respectively, in the figure) were hybridized separately to one of the three probes (C, 5′C, 3′C) indicated in the map at the bottom. The boxed numbers correspond to fragment sizes observed in the cloned b4Nb4 gene. Bands representing b4-related sequences without an internal *Eco*RI site hybridize with all three probes, whereas the 5′C and 3′C probes give a differential profile for sequences cut by *Eco*RI. An *Eco*RI digest of the five DNAs hybridized to the J probe gave the same pattern seen with the 5′C probe. Because of the importance of the *Eco*RI site within the coding region for our analysis, some confusion may result from the assertion by Heidmann and Rougeon (5) that the coding sequence probe from their cDNA clone did not have an *Eco*RI site, which suggests the possibility that their clone might represent a different b4 gene from ours. In fact, their published sequence (19) contains an *Eco*RI site at the position corresponding to residues 164–166 of their Fig. 4, and we believe that their cDNA is derived from the b4Nb4 gene represented by our genomic clones.

b5 gene may retain the *Eco*RI site, whereas the b6 and b9 sequences (Gln-Asn-Gly and Gln-Ser-Pro, respectively) are incompatible with an *Eco*RI recognition sequence at this position (b5, ref. 24; b6, ref. 25; b9, ref. 26). Therefore, it is of interest to know which of the multiple bands in the left panel of Fig. 4 represent fragments of κ sequences that have been split by *Eco*RI into two hybridizing bands and which bands represent intact sequences that lack an *Eco*RI site. This was analyzed by constructing the 5′C probe and 3′C probe (see map, Fig. 4), which represent sequences 5′ or 3′ to the *Eco*RI site of the cloned b4 gene. These probes were hybridized to blots identical to that of the left panel of Fig. 4, yielding results shown in the middle and right panels of Fig. 4.

One inference from these blots is that rabbits of all allotypes contain at least one κ sequence, represented by the 18-kb band, that is not internally cut by *Eco*RI, since the 18-kb band hybridizes to both the 5′ and 3′ probes. Based on the absence of the EcoRI site, these "18-kb" κ sequences probably do not represent genes that could encode the nominal allotype amino acid sequences determined for b4 or b5 κ chains. For convenience, we refer to these sequences as b4A sequences: b4Ab4, b4Ab5, b4Ab6, etc., depending on the DNA source.

In addition to these b4A sequences, b4, b4^v, b5, and b6 DNAs each contain at least one other κ sequence that *is* split by *Eco*RI. In b4 DNA, we can identify the 5′ 7.8-kb and 3′ 3.0-kb bands with the b4Nb4 gene represented by our clone. The identification of the *Eco*RI-split sequences in b4^v, b5, and b6 DNAs is less clear. The 11-kb band in b9 DNA may represent a sequence closely homologous to b4Nb4 except for the loss of the internal *Eco*RI site; thus, this band hybridizes to both the 5′C and 3′C probes and approximates the combined lengths of the 5′ and 3′ fragments of b4Nb4 (7.8 kb + 3.0 kb). For convenience, we refer to these unidentified sequences as b4B sequences: b4Bb4^v, b4Bb5, b4Bb6, and b4Bb9.

Is each b4-related sequence associated with a J region segment? To assess this question, a *Sau*3A fragment containing J2, the only J region known to be expressed, was hybridized with blots of *Eco*RI-digested DNA. The resulting pattern, indistinguishable from the one obtained with the 5′C probe (middle panel of Fig. 4), indicates that each b4A and b4B sequence has an associated J-like sequence. The other κ-related sequences detected only on the left panel by the coding sequence probe may be associated with J region segments with insufficient homology to the b4 J2 for hybridization to our J probe.

The blot probed with the *Pvu* II–*Ava* II fragment (left panel of Fig. 4) reveals bands that hybridize with neither the 5′C nor the 3′C probe. This may be explained by noting that the 5′C and 3′C probes are largely flanking sequence probes (see map, Fig. 4). Thus, although the sequences that hybridize only to the small *Pvu* II–*Ava* II probe may be homologous only to the b4 coding sequence (and/or to the short piece of 3′ untranslated region in this probe), the b4A and b4B sequences apparently share flanking sequence homology with the b4Nb4 gene. The existence of such flanking sequence homology was confirmed by experiments in which a probe from the J_κ–C_κ intron was found to yield an identical pattern of genomic Southern blot bands as a blot hybridized with the J probe (data not shown).

DISCUSSION

Expression of the b4 J Region Locus. We have detected five J-like gene segments in our cloned germline genomic b4 κ gene. This J cluster appears superficially similar to that of human and mouse in both inter-J spacing and distance from the C-region coding sequence. Because our sequence of about 500 bp 5′ to J1 and 800 bp 3′ to J5 reveals no other homologous sequences, we tentatively conclude that no further J sequences are present in this cluster.

However, of these J sequences only J2 corresponds to the known amino acid sequences observed in expressed b4 κ chains. A possible trivial explanation for the apparent suppression of the other J sequences is that the sequenced rabbit κ chains may have been unintentionally preselected for the presence of J2 by the choice of bacterial immunogens, just as J_H1 has been preselected in heavy chains from murine phosphorylcholine-binding antibodies (27).

On the other hand, a closer examination of the nucleotide sequences of the nonexpressed J sequences in the b4 cluster reveals that each may be intrinsically defective. The functional J regions of mouse and man are all found to have a conserved heptamer (C-A-C-T-G-T-G) and nonamer (G-G-T-T-T-T-T-G-T), or close approximation to these sequences, located 5′ to the J coding sequence and separated by a spacer of either 11–12 bp or 22–24 bp. These elements are thought to participate in V–J recombination along with complementary elements located 3′ of each V region. In addition, each expressed human or mouse J region sequence terminates on its 3′ end with a functional RNA splice site beginning "G-T . . ." that mediates the removal of the intervening sequence between J and C.

Fig. 2 compares these essential J characteristics in the J sequences found in our rabbit b4 cluster. The expressed J2 has normal nonamer and heptamer elements, separated by a 23-bp spacer, and an apparently normal RNA splice site at its 3′ end. However, J1 and J4 have abnormal spacer lengths of (respectively) 15 and 14 bp, which have never been observed for an expressed J sequence. J5 probably lacks a functional heptamer in that its heptamer departs from the consensus sequence in four of seven positions, more than the discrepancies observed in any functional J. Finally, J3 demonstrates the same RNA splice defect—substitution of "C-T . . ." for "G-T . . ." at the margin of the J–C intervening sequence—that was previously found in the corresponding J sequence of both mouse and rat. This observation suggests the surprising possibility that this nucleotide substitution may have antedated the evolutionary divergence of rabbit, mouse and rat.

b4-Related Sequences. The genomic Southern blots presented here clearly demonstrate that laboratory rabbits harbor several categories of sequences homologous to our b4Nb4 gene. Rabbits of all allotype strains harbor two sequences that share homology with b4Nb4 in J, C, and flanking regions. These are the b4A sequences (which apparently all lack an internal *Eco*RI site) and the b4B sequences (which include b4Nb4 and which—except in the case of b4Bb9—contain an internal *Eco*RI site). In addition to the b4A and b4B sequences, the rabbit DNAs also contain multiple additional sequences with homology detectable only to the C coding/3′-untranslated region of b4Nb4 and not to J2 or flanking regions.

What are the identities of these sequences? The bands detected by the coding sequence probe (*Ava* II–*Pvu* II; left panel of Fig. 4) probably include the nominal genes of the b5, b6, and b9 allotypes in the corresponding DNA samples because the amino acid homologies of these nominal κ chains to b4 κ (79%, 75%, and 67%, respectively) predict detectable homology at the nucleic acid level. However, the expected nominal gene bands cannot readily be distinguished by Southern blots from possible latent gene or pseudogene bands. Recent results from Heidmann and Rougeon (28) indicate that the b4Ab4 sequence encodes the κ2 isotype that was originally identified in *bas* rabbits (29) but is also expressed at low levels by other rabbits (30). It seems likely that the b4A sequences in the genomes of b5, b6, and b9 rabbits are also κ2 structural sequences.

The b4B bands may be interpreted in several ways in light of the latent allotype phenomenon; we mention two possibilities. (*i*) The b4B sequences may represent latent b4 structural genes in b5, b6, and b9 genomes, with respective nominal genes being encoded in the bands hybridizing exclusively to the coding sequence probe. (*ii*) The b4B sequences may represent the nominal allotype genes in the respective genomes. The presence of an *Eco*RI site in the b4Bb5 sequence and absence of same in b4Bb9 would be consistent with this interpretation because the amino acids at this site in b4Nb4 are preserved in the b5 amino acid sequence but altered in b9. (The published b6 amino acid sequence is incompatible with an *Eco*RI site at the homologous position; the *Eco*RI site observed in b4Bb6 could be explained by a nearby nonhomologous *Eco*RI site or by an amino acid sequence analysis error.) If the b4B sequences represent nominal allotype genes then the bands hybridizing exclusively to the coding sequence probe could be latent allotype genes. Alternatively, the latter bands may be pseudogenes and latent genes may be absent from the rabbit genomes that we have examined (because not every rabbit is observed to make latent allotype κ chains).

We are grateful to Ann Kuo for excellent technical assistance and to Virginia Shaw for her expert and patient editorial assistance in the preparation of this manuscript.

1. Strosberg, A. D., Hamers-Casterman, C., van der Loo, W. & Hamers, R. (1974) *J. Immunol.* **113**, 1313–1318.
2. Yarmush, M. L., Krutzsch, H. C. & Kindt, T. J. (1980) *Mol. Immunol.* **17**, 319–326.
3. McCartney-Francis, N. & Mandy, W. J. (1981) *Ann. Immun. Inst. Pasteur* **130C**, 115–131.
4. Kindt, T. J. & Yarmush, M. (1981) *CRC Crit. Rev. Immunol.* **2**, 297–348.
5. Heidmann, O. & Rougeon, F. (1982) *Cell* **28**, 507–513.
6. Wallace, B. E. & Salser, W. (1979) *Gene* **7**, 343–347.
7. Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3**, 2303–2309.
8. Rimm, D. L., Horness, D., Kucera, J. & Blattner, F. R. (1980) *Gene* **12**, 301–309.
9. Enquist, L. & Sternberg, N. (1979) in *Methods Enzymol.* **68**, 281–298.
10. Hohn, B. (1979) *Methods Enzymol.* **68**, 299–309.
11. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connel, C. & Quon, D. (1978) *Cell* **15**, 687–701.
12. Dreher, K. L., Emorine, L., Kindt, T. J. & Max, E. E. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4489–4493.
13. Tiemeier, C. D., Tilghman, S. M. & Leder, P. (1977) *Gene* **2**, 173–191.
14. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
15. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683–3687.
16. Messing, J. & Vieira, J. (1982) *Gene* **19**, 269–276.
17. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
18. Korn, L. J., Queen, C. L. & Wegman, M. N. (1978) *Proc. Natl. Acad. Sci. USA* **74**, 4401–4405.
19. Heidmann, O., Auffray, C., Cazenave, P. A. & Rougeon, F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5802–5806.
20. Chen, K. C. S., Kindt, T. J. & Krause, R. M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1995–1998.
21. Van Hoegarden, M. & Strosberg, A. D. (1978) *Biochemistry* **17**, 4311–4317.
22. Kabat, E. A., Wu, T. T. & Bilosfsky, H. (1979) *Sequences of Immunoglobulin Chains*, National Institutes of Health, Publ. No. 80-2008 (NIH, Bethesda, MD).
23. Yarmush, M. L., Gates, F. T., III, Dreher, K. L. & Kindt, T. J. (1981) *J. Immunol.* **126**, 2240–2244.
24. Chersi, A., Alexander, C. & Mage, R. (1980) *Mol. Immunol.* **17**, 1515–1523.
25. Emorine, L., Dutka, S., Paroutaud, P. & Strosberg, A. D. (1979) *Mol. Immunol.* **16**, 997–1004.
26. Farnsworth, V., Goodfleish, R., Rodkey, S. & Hood, L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1293–1296.
27. Gearhart, P. J., Johnson, D. N., Douglas, R. & Hood, L. (1981) *Nature (London)* **291**, 29–34.
28. Heidmann, O. & Rougeon, F. (1983) *EMBO J.* **2**, 437–441.
29. Kelus, A. S. & Weiss, S. (1977) *Nature (London)* **265**, 156–158.
30. Benammar, A. & Cazenave, P. A. (1982) *J. Exp. Med.* **156**, 585–595.