

SEQUENCE OF A cDNA ENCODING BASILEA KAPPA LIGHT
CHAINS (K2 ISOTYPE) SUGGESTS A POSSIBLE
RELATIONSHIP OF PROTEIN STRUCTURE TO LIMITED
EXPRESSION

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Occasional observations of kappa allotypes unexpected from a rabbit's pedigree (1-4) have raised questions of how many κ -like genes are within the rabbit genome and how their expression is regulated. Although light chain allotypes in several species are due to only a few amino acid differences (5-7), the rabbit κ allotypes b4, b5, b6, and b9 have unusually large sequence differences such that the constant regions of homozygous rabbits of these different b allotypes can be more different in sequence than the associated variable regions (8-13). In 1977, a new rabbit strain, Basilea, was developed from a b9 rabbit in which an apparent mutation resulted in failure to produce κ light chains (14). Although λ light chains were predominantly produced, further studies identified small amounts of a new κ -like light chain, the bas gene product (15-18). In breeding studies, the bas trait behaves as an allele at the b locus (14, 15); however, recent studies suggest that the light chain is a product of an isotypic gene (15, 20) (K2). Serological studies with anti-bas reagents have shown that some wild rabbits as well as some b9 rabbits produce bas light chains as well as two other b allotypes (15, 18). Recently, a gene encoding a κ light chain constant region (C_{κ}) was found in genomic libraries constructed from liver DNAs of b4 animals (19, 20). It was bas-like based on homology of the encoded protein to partial protein sequence of bas κ light chains (16). This bas-like gene was found within the genome of all rabbits studied along with the genes for the nominally expressed allotypes (19, 20). Three J-regions are associated with the bas-like gene (20). We report here the sequence of a cDNA clone from a homozygous Basilea rabbit that encodes a variable, J, and constant region of the bas κ type. This sequence proves that the nonexpressed bas-like C_{κ} genes (19, 20) are essentially the same in coding sequence as that expressed by the bas rabbit.

Materials and Methods

Rabbits. A colony of Basilea rabbits was developed in our breeding facilities at the National Institutes of Health from stock kindly donated to us by Dr. Andrew Kelus, Basel N. M.-F. was supported by National Research Service Award, AI 06435-02. Reprint requests should be addressed to Dr. Mage.

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mRNA. mRNA was prepared from splenic lymphocytes of an a2a3, basbas, de12,15 (haplotype F/G (21)) rabbit that had been infected with *Trypanosoma equiperdum*. The exact protocols for RNA preparation and immunization have been described previously (22).

cDNA. cDNA was prepared using reverse transcriptase, DNA polymerase, and S1 nuclease as previously described (22). Poly C tails were added using terminal deoxynucleotidyl transferase (23) and the tailed cDNA was inserted into the Pst I site of pBR322. *E. coli* strain MC1061 was transformed and selected on tetracycline-containing media (24). Individual colonies were selected by colony hybridization (25) using a nick-translated full length b9 cDNA as a probe (8).

DNA Sequencing. All sequencing data were obtained using the method of Maxam and Gilbert (26). DNA was either 5' labeled using [γ - 32 P] ATP and polynucleotide kinase or the 5' extensions of fragments were "filled in" using [α - 32 P] dCTP or [α - 32 P] dGTP and the large fragment of DNA polymerase. 3' end labeling with 32 [P] ddATP and terminal transferase was also used. Sequence data were obtained from both strands of clone κ bas-3C8 and all restriction sites labeled for sequencing were themselves sequenced from other sites. Additional sequence data were obtained on one strand of clone κ bas-5F4 for the terminal 83 bases of the 3' UT region not encoded by clone κ bas-3C8.

Results

Fig. 1 shows the nucleic acid and deduced amino acid sequence of clone κ bas-3C8. The leader (*L*) and variable region (*V_x*) through amino acid 95A is compared to that previously found in a b9 cDNA clone κ b9-17D9 (8). The J region of clone κ bas-3C8 is compared to the J1 bas sequence (20) and the constant region is compared to the bas-like constant region sequence (19, 20). We refer to these J and C_x genes and their potential protein products as bas-N4 since they were isolated from the DNAs of rabbits with nominal allotype b4.

The leader and V region of clone κ bas-3C8 (amino acid positions -14 through 95A) is 93% homologous to that of the κ b9-17D9 cDNA clone from a b9-expressing animal for the 334 bases compared (Fig. 1). As in clone κ b9-17D9, codons for cysteines are found at positions 23 and 88, permitting the formation of the intradomain disulfide bond. In addition, in both of these V_x regions there is a codon for Arg (CGT) instead of the typical Cys (TGT) at position 80, a finding that will be discussed below.

The J region identified in clone κ bas-3C8 differs from those associated with the other b allotypes. Each of the rabbit b allotypes appears to be expressed with a different set of J-regions (8, 12, 20, 28). Of the three J regions associated with the bas-N4 gene, J1 is identical to our sequence with the exception of the codon at the 5' junction and one additional nucleotide.

The constant region of clone κ bas-3C8 is virtually identical to the bas-N4 gene (19). The one change is at amino acid position 204 where we find a codon for Leu (CTG) in place of the reported Pro (CCG). The change also falls within the reported Pvu I site of the bas N4 sequence (19) and as expected our cDNA is resistant to digestion with this enzyme. The 3' UT regions of clone κ bas-3C8 and the bas N4 gene are identical with the exception of two single base changes. It is noteworthy that the poly A tail in this cDNA is found only 12 or 13 bases away from the AATAAA polyadenylation signal, whereas in b4 it was found to be 17 bases away (11) and in b5 (12) and b9 (8) it was found 19 bases away.

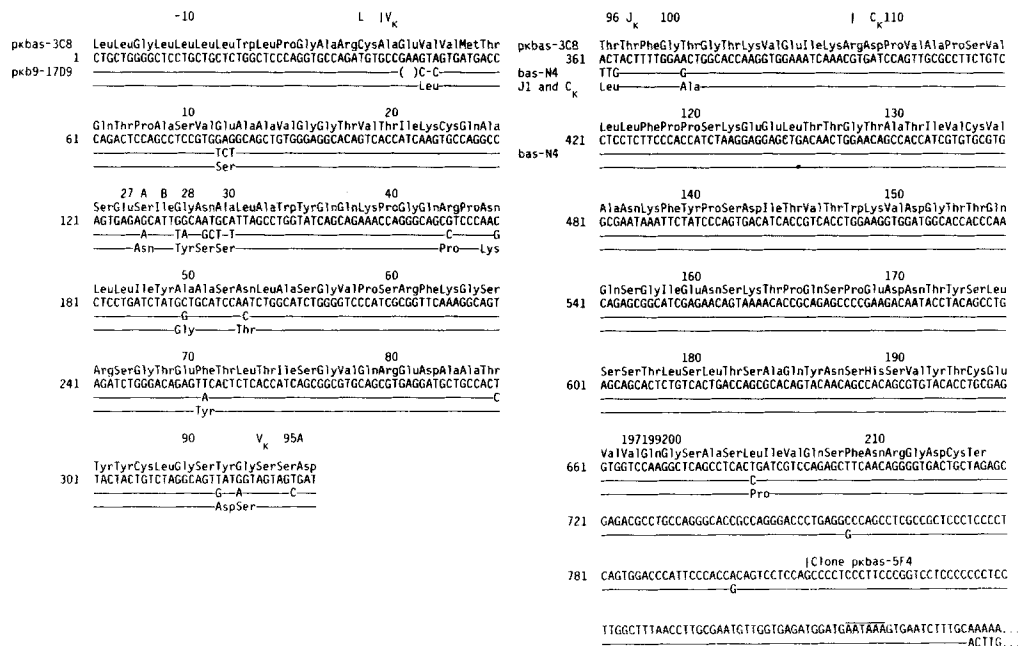


FIGURE 1. Fig. 1 shows the sequence of *pkbas-3C8* cDNA and the deduced protein sequence encoded by this cDNA clone that was isolated from splenic mRNA of a Basilea rabbit. Amino acids are numbered above according to the standard system of Kabat et al. (27) and bases are numbered sequentially on the left side of the lines. The leader (L) and variable region (V_K) sequences are compared to the sequence of clone *pkb9-17D9* isolated from mRNA of a homozygous b9 rabbit (8). The J and constant region (C_K) sequences of *pkbas-3C8* are compared to one of three J regions (J1) (20) and to a bas-like C_K gene (*bas-N4*) found within genomic DNAs from two different b4 rabbits (19, 20). The last 83 bases of our bas sequence are from a second bas cDNA clone *pkbas-5F4* which extends to the poly(A) tail. The base at position 69, which we assign as adenine, was difficult to determine on both strands of *pkbas-3C8*. A line has been drawn above the AATAAA polyadenylation signal sequence.

Discussion

The observation of: (a) the bas mutation (14); (b) a bas-like gene in the DNA of all rabbits studied (19, 20, 28, 29); and (c) small quantities of bas protein produced by some but not all normal animals expressing other b allotypes (15–18), raises the questions of what molecular change(s) took place in the bas mutant to suppress b9 kappa chain expression and permit the expression and identification of the bas isotype, and why it is that if all rabbits possess the bas gene, they do not produce substantial quantities of bas light chains.

A pertinent observation is that the V region in our bas clone *pkbas-3C8* is unusual in lacking the "invariant" cysteine found at position 80 in almost all rabbit κ light chains. Most rabbit κ chains have three intrachain disulfide bonds, two that are intradomain as in other species and one, an unusual bond that joins the variable and constant domains, usually through positions 80 and 171. In some b9 light chains, there is no Cys at 80 but there is a Cys at J-region position 108 that can bond to the constant region Cys 171 (8). However, the three available J_K sequences of *bas-N4* do not encode a Cys at position 108 (20). The

bas constant region lacks the extra Cys at position 171. Thus, if the typical rabbit V_{κ} region encoding Cys at position 80 is rearranged and expressed with C_{κ} bas, the light chain could have a free sulfhydryl group. This may lead to a nonfunctional or poorly functional molecule. It has been suggested that assembly of heavy and light chains signals termination of light chain gene rearrangements, perhaps by light chain displacement of a heavy-chain-binding protein in pre-B cells (30). If most bas (K2) light chains cannot adequately interact with heavy chains and supply this shut-off signal, other V_L to J_L gene rearrangements may continue until a light chain that is assembled into a functional immunoglobulin is produced. We postulate that in animals normally producing small amounts of bas light chains, pre-B cells expressing other types of κ and λ light chains develop into surface immunoglobulin positive B cells more frequently than do pre-B cells expressing only the bas type. We do not yet know what change(s) occurred in the Basilea mutant that led to little or no production of b9. The production of bas (K2) light chains by these rabbits may result from expansion of the few bas B cells producing light chains encoding V_{κ} regions without the Cys at 80. These V_{κ} genes probably represent a relatively small family encoding related sequences that we have now found in b9 (8) and bas rabbits.

We present here the sequence of a cDNA encoding a light chain that was constructed from mRNA of a bas rabbit. The J region equivalent to that expressed in our clone ($p\kappa$ bas-3C8) has been found associated with the bas-like C_{κ} gene in genomic b4 DNA (20). In the genome the signal sequences for DNA rearrangement and RNA processing associated with this J seem to be intact (20). The constant region of the expressed molecule differs from that encoded by the bas-like gene found in b4 animals (19, 20) only by the codon for Leu (CTG) replacing Pro (CCG) at amino acid position 204. This amino acid is entirely deleted from b4 chains and is Pro (CCG) in b5 (12) and Ala (GCG) in b9 (8). Thus, it is very unlikely that this single difference prevents expression of the bas gene in b4 animals. There are probably several different levels of regulation at which the relative expression of b allotypes (K1) in heterozygous rabbits, and relative proportions of K1 and K2 isotypes are affected. Comparisons of the noncoding regions surrounding the exons of bas-like genes (20) in animals of different phenotypes may reveal some significant differences. It is known that even a single base change can completely prevent secretion of an immunoglobulin light chain (32). In animals producing the Basilea (K2) light chain, the absence of cysteine at position 171 in the constant region, and resulting potential for light chains with a free variable region SH, is at present, implicated as a likely reason for its relatively low expression.

Summary

We present the complete sequence of a cDNA encoding rabbit immunoglobulin kappa light chains of the Basilea isotype (K2). Although all rabbits seem to possess a K2 constant region gene, expression of this gene in most rabbits is minimal if present at all. Even in Basilea rabbits the majority of expressed immunoglobulins are of lambda type. We find that the sequence of our Basilea cDNA constant region and the sequence of a "silent" K2 gene from b4 rabbits (bas-N4) are almost identical. The bas (K2) isotype lacks cysteine at position 171

in the constant region that is present in all K1 constant regions and usually forms an interdomain disulfide bond, with a cysteine at position 80 of the variable region. We postulate that one factor contributing to the low expression of the bas (K2) isotype could be a paucity of V_{κ} regions lacking cysteine at position 80. If a typical rabbit V_{κ} encoding Cys at position 80 is rearranged and expressed with the K2 isotype, B cells with mRNAs encoding light chains with free sulfhydryl groups would result. These cells may fail to form functional immunoglobulin receptors. Only a small subset of rabbit variable regions that lack the cysteine at position 80 would rearrange and encode K2 light chains lacking a free sulfhydryl group.

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References

1. Strosberg, A. D. 1977. Multiple expression of rabbit allotypes: the tip of the iceberg? *Immunogenetics*. 4:499.
2. Kindt, T. J., and M. Yarmush. 1981. Expression of latent immunoglobulin allotypes and alien histocompatibility antigens: relevance to models of eukaryotic gene regulation. *CRC Crit. Rev. Immunol.* 2:297.
3. McCartney-Francis, N., and W. J. Mandy. 1981. Control of latent allotype expression by rabbit splenocytes. *J. Immunol.* 127:352.
4. Roux, K. H. 1983. Rabbit latent allotypes: current status and future directions. *Surv. Immunol. Res.* 2:342.
5. Mage, R., R. Lieberman, M. Potter, and W. D. Terry. 1973. Immunoglobulin allotypes. In *The Antigens*. Vol. I. M. Sela, editor. Academic Press, New York. p. 299.
6. Sheppard, H. W., and G. A. Gutman. 1981. Allelic forms of rat kappa chain genes: evidence for strong selection at the level of nucleotide sequence. *Proc. Natl. Acad. Sci. USA.* 78:7064.
7. Arp, B., M. D. McMullen, and U. Storb. 1982. Sequences of immunoglobulin $\lambda 1$ genes in a $\lambda 1$ defective mouse strain. *Nature (Lond.)*. 298:184.
8. McCartney-Francis, N., R. M. Skurla, Jr., R. G. Mage, and K. E. Bernstein. Kappa chain allotypes and isotypes in the rabbit: cDNA sequences of clones encoding b9 suggest an evolutionary pathway and possible role of the interdomain disulfide bond in quantitative allotype expression. *Proc. Natl. Acad. Sci. USA.* In press.
9. Kindt, T. J. 1975. Rabbit immunoglobulin allotypes: structure, immunology, and genetics. *Adv. Immunol.* 21:35.
10. Mage, R. G. 1983. Rabbit immunoglobulin allotypes. In *Handbook of Immunology*. 4th ed. D. M. Weir, L. A. Herzenberg, C. C. Blackwell, and C. C. Herzenberg, editors. Blackwell Scientific Publications, Edinburgh. In press.
11. Heidmann, O., C. Auffray, P-A Cazenave, and F. Rougeon. 1981. Nucleotide sequence of constant and 3' untranslated regions of a κ immunoglobulin light chain mRNA of a homozygous b4 rabbit. *Proc. Natl. Acad. Sci. USA.* 78:5802.
12. Bernstein, K. E., R. Skurla, and R. G. Mage. 1983. The sequences of rabbit κ light chains of b4 and b5 allotypes differ more in their constant regions than in their 3' untranslated regions. *Nucleic Acids Res.* 11:7205.
13. Farnsworth, V., R. Goodflesh, S. Rodkey, and L. Hood. 1976. Immunoglobulin allotypes of rabbit kappa chains: polymorphism of a control mechanism regulating

- closely linked duplicated genes? *Proc. Natl. Acad. Sci. USA.* 73:1293.
14. Kelus, A. S., and S. Weiss. 1977. Variant strain of rabbits lacking immunoglobulin κ polypeptide chain. *Nature (Lond.)* 265:156.
 15. Good, P. W., R. Notenboom, S. Dubiski, and B. Cinader. 1980. BASILEA rabbit immunoglobulins: detection and characterization by specific alloantiserum. *J. Immunol.* 125:1293.
 16. Garcia, I., D. C. Brandt, A. Benammar, P.-A. Cazenave, and J.-C. Jaton. 1982. BASILEA rabbits express two types of immunoglobulin light chains: λ and κ -like. *Proc. Natl. Acad. Sci. USA.* 79:4391.
 17. Weiss, S., I. Garcia, J.-C. Jaton, and A. S. Kelus. 1982. Allotypes in Basilea rabbits. *Eur. J. Immunol.* 12:170.
 18. Benammar, A., and P. A. Cazenave. 1982. A second rabbit kappa isotype. *J. Exp. Med.* 156:585.
 19. Heidmann, O., and F. Rougeon. 1983. Multiplicity of constant kappa light chain genes in the rabbit genome: a b4b4 homozygous rabbit contains a κ -bas gene. *EMBO (Eur. Mol. Biol. Org.) J.* 2:437.
 20. Emorine, L., and E. E. Max. 1983. Structural analysis of a rabbit immunoglobulin κ 2 J-C locus reveals multiple deletions. *Nucleic Acids Res.* In press.
 21. Mage, R. G., S. Dray, A. Gilman-Sachs, C. Hamers-Casterman, R. Hamers, W. C. Hanly, T. J. Kindt, K. L. Knight, W. J. Mandy, and J. Naessens. 1982. Rabbit heavy chain haplotypes—allotypic determinants expressed by V_H - C_H recombinants. *Immunogenetics.* 15:287.
 22. Bernstein, K. E., A. Pavirani, C. Alexander, F. Jacobsen, L. Fitzmaurice, and R. Mage. 1983. Use of *trypanosoma equiperdum* infected rabbits as a source of splenic mRNA; construction of cDNA clones and identification of a rabbit μ heavy chain clone. *Mol. Immunol.* 20:89.
 23. Otsuka, A. 1981. Recovery of DNA fragments inserted by the 'tailing' method: regeneration of Pst I restriction sites. *Gene.* 13:339.
 24. Hutchinson, K., and H. Halvorson. 1980. Cloning of a randomly sheared DNA fragment from a ϕ 105 lysogen of *Bacillus subtilis* identification of prophage-containing clones. *Gene.* 8:267.
 25. Thayer, R. 1979. An improved method for detecting foreign DNA in plasmids of *Escherichia coli*. *Anal. Biochem.* 98:60.
 26. Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* 65:498.
 27. Kabat, E. A., T. T. Wu, H. Bilofsky, M. Reid-Miller, and H. Perry. 1983. Sequences of proteins of immunological interest: tabulation and analysis of amino acid and nucleic acid sequences of precursors, V-regions, C-regions, J-chain, β_2 -microglobulins, major histocompatibility antigens, Thy-1, complement, C-reactive protein, thymopoietin, post-gamma globulin, and α 2-macroglobulin. *US DHHS, PHS, NIH.*
 28. Emorine, L., K. Dreher, T. J. Kindt, and E. E. Max. 1983. Rabbit immunoglobulin κ genes: structure of a germline b4 allotype J-C locus and evidence for several b4-related sequences in the rabbit genome. *Proc. Natl. Acad. Sci. USA.* 80:5709.
 29. Heidmann, O., and F. Rougeon. 1982. Multiple sequences related to a constant-region kappa light chain gene in the rabbit genome. *Cell.* 28:507.
 30. Wabl, M., and C. Steinberg. 1982. A theory of allelic and isotypic exclusion for immunoglobulin genes. *Proc. Natl. Acad. Sci. USA.* 79:6976.
 31. 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.
 32. Wu, G. E., N. Hozumi, and H. Murialdo. 1983. Secretion of a λ 2 immunoglobulin chain is prevented by a single amino acid substitution of its variable region. *Cell.* 33:77.