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Structural differences in a single gene encoding the V_κSer group of light chains explain the existence of two mouse light-chain genetic markers

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ABSTRACT **Two phenotypic markers of mouse immunoglobulin κ light chains, the I_B-peptide marker and the Ef1[a] isoelectric focusing marker, are expressed by the C58/J, AKR/J, RF/J, and PL/J strains (called expressor strains) but not by BALB/c and most inbred strains. Expression is linked to the κ light-chain locus and the *Lyt-2/Lyt-3* genes on chromosome 6. Light chains bearing these markers belong to a group of variable region κ chain (V_κ) regions called V_κSer, which has a serine amino terminus and a framework 1 region not observed to date among BALB/c light chains. Southern hybridization of genomic DNA with a V_κSer-specific cDNA probe has demonstrated a single strongly hybridizing DNA fragment in all strains of mice tested. Characteristic restriction enzyme polymorphisms define the V_κSer alleles of expressor (*Igk-VSer*[a]) and nonexpressor (*Igk-VSer*[b]) strains. In the present study, the unrearranged *V_κSer* gene and its flanking regions from an expressor (C.C58) and nonexpressor (BALB/c) strain have been cloned and their nucleotide sequences determined. The C.C58 *V_κSer* gene isolated (the *Igk-VSer*[a] allele) was shown to code for the two phenotypic markers described. While the nucleotide sequence of the BALB/c coding region (the *Igk-VSer*[b] allele) shows 97% identity with the C.C58 gene, single nucleotide substitutions lead to structural changes in the encoded protein which render it I_B-negative and Ef1[a]-negative. These differences alone can explain the failure of strains containing the BALB/c allele to express these κ-chain phenotypic markers. Also, the BALB/c gene contains a single substitution in a conserved octamer sequence ≈100 nucleotides upstream of the coding region, which could affect its expression. Finally, the C.C58 allele contains a BAM5/R repetitive DNA element ≈1200 nucleotides upstream of the coding regions that is not present in BALB/c. This element gives rise to the *Eco*RI and *Bam*HI restriction enzyme polymorphisms, which distinguish the *Igk-VSer*[a] and *Igk-VSer*[b] alleles.**

Genes coding for the variable (V) regions of antibody κ light (L) chains are assembled by the joining at the DNA level of a segment encoding the leader and amino-terminal 95 amino acids (the V_κ segment) to one of several joining (J) regions that encode the remainder of the V region and lie 5′ to an exon encoding the constant region (see ref. 1 for review). Functional heavy (H)-chain V-region genes similarly involve joining of separately encoded gene segments in cells destined to produce antibodies. The repertoire of antibody combining sites that an individual can produce is determined in part by the number and structure of the germ-line gene segments that participate in such rearrangements. Differences among individuals in structure and/or expression of particular *V* gene segments could lead to differences in the phenotypes of antibody molecules produced.

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A number of phenotypic genetic polymorphisms have been shown to map to immunoglobulin H and L chain loci (see ref. 2 for review). In several instances, the expression of strain-specific idiotypes has been shown to be due to differences among strains in the structure or repertoire of particular V_H gene segments (3–5). A number of immunoglobulin κ L-chain polymorphisms have been described (6–12), and in several instances Southern hybridization analyses have provided evidence that mice exhibit restriction enzyme polymorphisms involving V_κ gene segments (13–15).

The present study explores the molecular genetic basis for two mouse V_κ polymorphisms, the I_B-peptide marker (16) and the Ef1[a] isoelectric focusing marker (17). Both have been shown to be manifestations of the production of a characteristic group of κ-chain V regions (called V_κSer) by several strains of mice (C58/J, AKR/J, PL/J, and RF/J) but not by BALB/c and most other strains (18). Expression of these and other κ polymorphisms maps to the κ locus, which is closely linked to the *Lyt-2* and *Lyt-3* genes on chromosome 6 (19). Gottlieb *et al.* (18) have identified myeloma tumors producing κ chains corresponding to the I_B-peptide marker and Ef1[a] polymorphism in the C.C58 and C.AKR strains, which differ from BALB/c only in the region of the κ and *Lyt-2/Lyt-3* loci (20). These myelomas permitted isolation of a cDNA probe specific for the V_κSer group, and Southern hybridizations with this probe provided evidence for a single strongly hybridizing V_κSer-related gene in liver DNA of all strains tested (13). Restriction enzyme polymorphisms were observed among the strains tested, which correlated completely with expression or nonexpression of the I_B-peptide and Ef1[a] phenotypic markers. Expressor strains contained the *Igk-VSer*[a] allele and nonexpressor strains contained the *Igk-VSer*[b] allele (13, 21).

In the present studies, the V_κSer-related genes of the expressor strain, C.C58, and the nonexpressor strain, BALB/c, have been cloned from phage libraries of liver DNA. Comparison of their nucleotide sequences demonstrates differences in their coding and 5′ flanking regions, which explain the differences observed in expression of the I_B-peptide and Ef1[a] phenotypic markers. Findings also bear generally upon the nature and origins of polymorphisms in mouse V_κ genes.

MATERIALS AND METHODS

Cloning of C.C58 and BALB/c *V_κSer* Genes. Liver DNA from C.C58 (20) and BALB/cAn mice raised in our colony was extracted according to a modification of the method of Maniatis *et al.* (22). DNA was subjected to partial digestion with *Mbo* I (New England Biolabs), and fragments 10–20 kilobases long were cloned into the *Bam*HI site of the λ phage vector EMBL3 (Promega Biotec, Madison, WI) according to

Abbreviations: V and J, variable and joining regions of immunoglobulin; H chain and L chain, heavy chain and light chain.

the procedure of Frischauf *et al.* (23). Phage plaques (3–4 × 10^5) were screened with a 200-nucleotide cDNA probe specific for the V_κSer group (13). Clones that hybridized with this probe were grown and DNA was purified according to Maniatis *et al.* (22). Restriction mapping was performed following standard procedures.

Nucleotide Sequence Determination. Fragments of the EMBL3 genomic clones were subcloned into the vectors M13mp8, M13mp18, and M13mp19 (24), and their nucleotide sequences were determined by the dideoxynucleotide chain-termination method (25). Deletions were made in some of the fragments by using BAL-31 nuclease (26) before subcloning.

RESULTS

Partial *Mbo* I libraries of C.C58 and BALB/c liver DNA in the λ vector EMBL3 were screened with a 200-base-pair cDNA probe specific for the V_κSer subgroup. Restriction maps of clones isolated are shown in Fig. 1. The sizes of the *Bam*HI, *Eco*RI, and *Hin*dIII fragments containing the V_κSer coding sequences in these clones are consistent with the sizes detected in Southern hybridizations of liver DNA from the two strains (13). Thus, the *V_κSer* genes isolated from the two strains appear to be the same genes distinguished previously by restriction polymorphisms that correlated with expression of V_κSer-related phenotypic markers.

As shown in Fig. 1, the restriction maps of the C.C58 and BALB/c clones 3′ to the coding segments are very similar, but they diverge completely in the 5′ flanking regions. To explore the basis for differences in expression of V_κSer-related κ chains by the two strains, the nucleotide sequences of the C.C58 and BALB/c *V_κSer* genes and >1400 base pairs of flanking DNA were determined as described in Fig. 2 and *Materials and Methods*. Results of nucleotide sequence analyses are presented in Fig. 3.

DISCUSSION

The V_κSer-specific probe used to isolate the C.C58 and BALB/c genomic clones shown in Fig. 1 was the 5′ 200 base pairs of a V_κSer cDNA clone isolated from the C.C58 M75 myeloma (13). The 5′ untranslated, leader, and V segment sequences present in the EM3.C58 7 clone differ by only 3 single base-pair substitutions (positions 411, 544, and 552) (Fig. 3) from the corresponding region of the C.C58 M75 L chain cDNA clone, p9(35) (13). This strongly suggests that the C.C58 gene present in the EM3.C58 clone is the V_κSer gene, which is rearranged and expressed by the C.C58 M75 myeloma. This conclusion is supported by nucleotide sequence analysis of a clone containing the expressed C.C58 M75 myeloma L-chain gene (unpublished data).

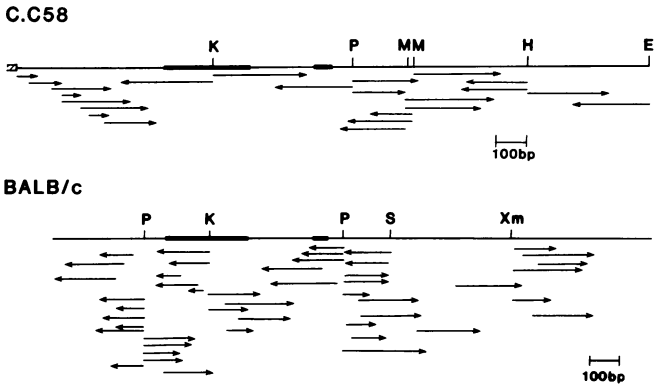

FIG. 2. Strategy for determination of the nucleotide sequences of C.C58 and BALB/c *V_κSer* genes and flanking regions. Fragments of clones EM3.C58-7 and EM3.BALB-1 were subcloned into phage M13mp8, M13mp18, and M13mp19, and their nucleotide sequences were determined as described in *Materials and Methods*. Coding sequences are indicated by solid bars and phage sequences are indicated by hatched bars. Restriction enzyme cutting sites used for subcloning are as follows: K, *Kpn* I; P, *Pvu* II; M, *Mbo* I; H, *Hin*dIII; E, *Eco*RI; S, *Sac* I; Xm, *Xmn* I. bp, Base pairs.

Comparison of the nucleotide sequence of the C.C58 V_κSer genomic clone with that of BALB/c reveals extensive homology in the genes and in their flanking regions, which is consistent with the interpretation that they are alleles (Fig. 3). In only one other case, that of the mouse T15 V_H gene family (28), has the structure of allelic immunoglobulin genes been compared. A single base substitution leads to a conservative isoleucine to valine replacement in the leader segment, and as in C.C58, the amino terminus of the mature L chain encoded by the BALB/c V_κSer gene is serine. The latter is unique among κ chains, which generally have amino-terminal aspartic acid, asparagine, or glutamic acid (29). Twelve base substitutions are observed in the V coding segment, nine of which result in differences in the amino acid sequences of the germ-line genes. As shown in Fig. 4, all of the amino acid substitutions are in the framework (FR1), complementarity determining region 1 (CDR1), FR3, and CDR3 regions. The protein product of the BALB/c *V_κSer* gene has never been observed among myeloma or other antibodies, although there is evidence (see below) that the gene can be rearranged *in vivo*.

Several of the amino acid substitutions observed in the BALB/c gene could explain the failure of BALB/c mice to express κ-chain phenotypic markers associated with the C.C58 *V_κSer* gene. The replacement of methionine-21 of C.C58 with isoleucine in BALB/c (Fig. 3) can explain the failure of the latter strain to express the I_B-peptide pheno-

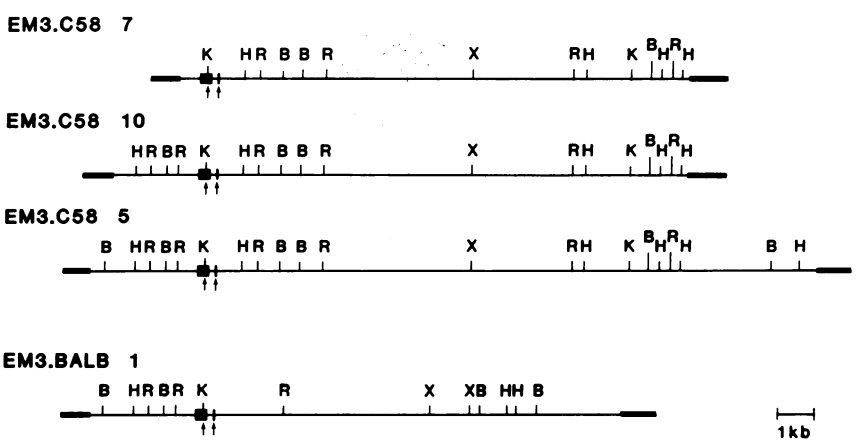

FIG. 1. Restriction maps of liver DNA fragments containing the *V_κSer* genes of C.C58 (clones EM3.C58-5, -7, and -10) and BALB/c (clone EM3.BALB-1) cloned into the *Bam*HI site of the λ phage vector EMBL3 (see *Materials and Methods*). Coding sequences are indicated by arrows. Transcription of the gene is from right to left. Restriction enzyme cleavage sites are as follows: K, *Kpn* I; H, *Hin*dIII; R, *Eco*RI; B, *Bam*HI; X, *Xba* I. kb, Kilobase.

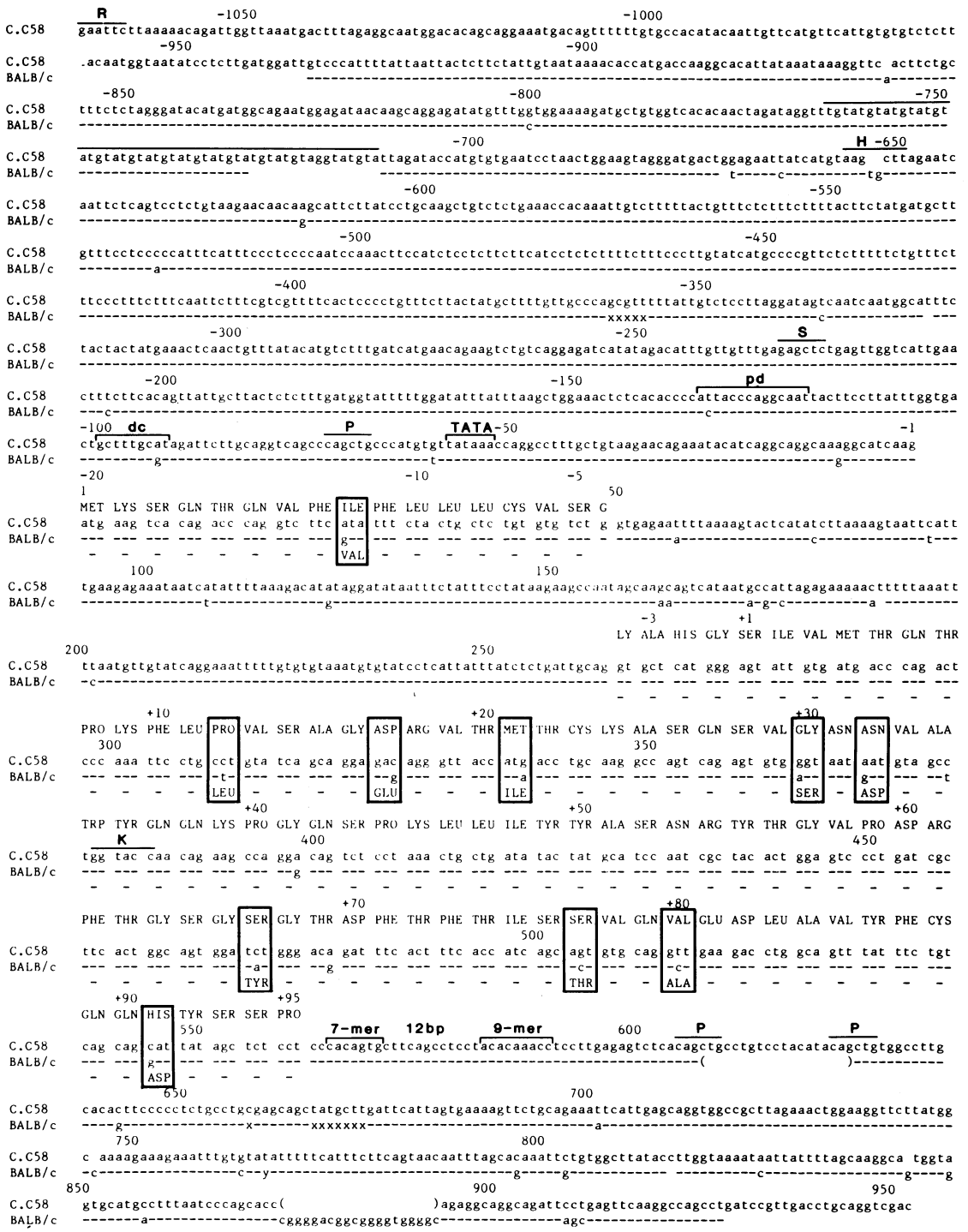

FIG. 3. Comparison of the nucleotide sequences and encoded amino acid sequences of the unrearranged $V_\kappa Ser$ genes of C.C58 and BALB/c mice. Nucleotide sequences were determined from the EM3.C58 7 and EM3.BALB 1 clones (see Figs. 1 and 2 and *Materials and Methods*). The first nucleotide of the ATG codon at which translation is initiated is designated nucleotide position 1. The nucleotide sequences from position −1070 to −936 and from 930 to 952 of the BALB/c gene have not been determined. Amino acids of the L1 leader segment are numbered −20 to −5, and those of the L2-V coding segment are numbered −3 to +95. In the coding regions, the nucleotide sequences are represented as triplets with the amino acid encoded by the C.C58 sequence shown directly above. Positions where the BALB/c nucleotide and amino acid sequences are identical to C.C58 are indicated by dashes. Unidentified bases and bases identified only as pyrimidines are designated x and y, respectively. Deleted bases are left blank. Amino acid replacements resulting from nucleotide substitutions in the BALB/c gene are shown beneath the BALB/c nucleotide sequence, and all positions containing amino acid differences are enclosed in boxes. The *dc, pd* sequences of Falkner and Zachau (27), the presumed *TATA* box, and the 7-mer and 9-mer sequences thought to be involved in gene rearrangement are indicated by a bracket over the C.C58 nucleotide sequence. Recognition sites for the relevant restriction enzymes are indicated by a bar over the C.C58 nucleotide sequence and are abbreviated as follows: R, *Eco*RI; H, *Hin*dIII; S, *Sac* I; P, *Pvu* II; K, *Kpn* I. The TGTA repetitive sequence in the neighborhood of nucleotide −750 is also indicated by a bar over the sequence. The sequence of the BALB/c gene in the region enclosed in parentheses (positions 608–626) was not determined, probably because the sequencing strategy making use of the *Pvu* II site resulted in loss of the small *Pvu* II/*Pvu* II fragment. The sequence of the C.C58 gene in the region enclosed in parentheses (positions 874–893) could not be determined by the dideoxynucleotide method.

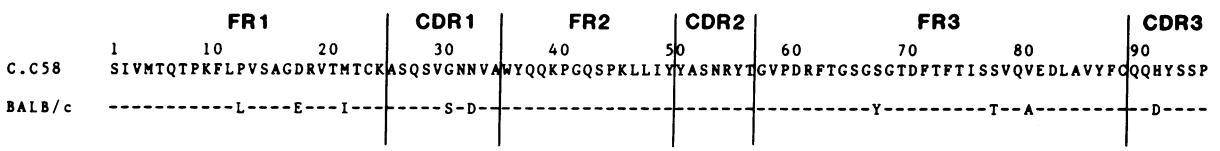

FIG. 4. Comparison of the predicted amino acid sequences of positions 1–95 of the L-chain products encoded by the C.C58 and BALB/c $V_\kappa Ser$ genes. Positions where the BALB/c product is the same as C.C58 are indicated by dashes. Frameworks 1, 2, and 3 (FR1, FR2, and FR3, respectively) and complementarity determining regions 1, 2, and 3 (CDR1, CDR2, and CDR3, respectively) are designated according to Kabat *et al.* (29). The one-letter code for amino acids is used (30).

typic marker. That marker, detected in two-dimensional tryptic peptide maps of fully reduced and [^{14}C]iodoacetic acid-alkylated normal serum immunoglobulin L chains (16), was shown to be due in part to the peptide Val-Thr-Met-Thr-Cys-Lys, which includes methionine-21 and is present in the C.C58 M75 myeloma κ chain (18). Substitution of isoleucine for methionine in this position results in a peptide that does not migrate as the I_B-peptide marker (H. C.-W. Tsang and P.D.G., unpublished results). Similarly, substitution of both asparagine-32 and histidine-91 of the C.C58 gene with aspartic acid in BALB/c (Fig. 3) would explain the failure of BALB/c to express the Ef1^a phenotypic marker detected by Gibson (17) by isoelectric focusing of fully reduced and [^{14}C]iodoacetamide-alkylated normal serum immunoglobulin L chains. The BALB/c $V_\kappa Ser$ gene product would migrate to a position on an isoelectric focusing gel more acidic than the C.C58 protein, and BALB/c would therefore lack a protein at the position of the Ef1^a marker. Thus, the putative BALB/c gene product contains amino acid substitutions that render its protein product both I_B-negative and Ef1^a-negative. Lacking a C.C58-like $V_\kappa Ser$ gene, the BALB/c strain is phenotypically negative for these markers.

These results are analogous to those of Bothwell *et al.* (4) and Loh *et al.* (5) in studies of the V_H gene used by C57BL/6 mice to produce antibodies against the (4-hydroxyl-3-nitrophenyl)acetyl (NP) hapten. These antibodies bear an idiotype, NP^b, not produced by BALB/c mice. Genes were found in BALB/c that were closely related to the C57BL/6 V_H gene used in NP^b antibodies. However, the BALB/c genes contained substitutions likely to make their gene products unsuitable for anti-NP antibodies. While the antigen recognized by immunoglobulins bearing V_κSer L chains in C.C58 mice is not known, the substitutions present in the BALB/c $V_\kappa Ser$ gene, particularly in the complementarity determining regions, may render its product unsuitable for the same immune response.

As stated earlier, it is not known whether BALB/c mice express the product of their $V_\kappa Ser$-related gene in antibodies. That the BALB/c $V_\kappa Ser$ gene can be rearranged *in vivo* is demonstrated by comparison of its nucleotide sequence with that of a fragment of a rearranged V_κ gene isolated by Nishi *et al.* (31). With the exception of a silent *t* to *c* substitution at position 538 (Fig. 3), this fragment (clone 4; ref. 31) is identical in sequence with the BALB/c $V_\kappa Ser$ gene segment from the *Kpn* I site (Fig. 3) to the point of V–J joining. Although the means of isolation of this gene fragment precluded obtaining sequence data 5′ of the *Kpn* I site, the near perfect identity of the sequences 3′ to that site strongly indicates that clone 4 represents rearrangement of the BALB/c $V_\kappa Ser$ gene.

While no myeloma or hybridoma L chain with the characteristic amino-terminal serine residue and framework substitutions of the BALB/c $V_\kappa Ser$ gene has been identified among the large number of BALB/c myeloma and hybridoma chains examined (29, 32, 33), it is possible that an insufficient number of BALB/c proteins have been studied at random to allow its detection. Among 200 myelomas studied in the C.C58 and C.AKR strains, eight belonged to the V_κSer group (18), consistent with the level of expression of the I_B-peptide marker in serum immunoglobulin L chains (16). It is possible that the rearranged and expressed BALB/c $V_\kappa Ser$ gene is represented less frequently in the B-cell pool than is the C.C58 gene, and that this may account for the failure to date to detect its product. The level of normal expression of the BALB/c $V_\kappa Ser$ gene is being assessed by producing hybridomas of lipopolysaccharide-stimulated BALB/c spleen cells and screening by the method of Manser and Gefter (34) for clones producing V_κSer-related mRNA.

It is possible that failure to detect the BALB/c $V_\kappa Ser$ gene product to date might be due to defects in nucleotide sequences required for its rearrangement and/or expression. As shown in Fig. 3, the region of the BALB/c gene immediately 3′ to the coding segment, which is thought to be involved in specific joining to the J segment (1), is identical in sequence to the functional C.C58 gene. Both genes also contain identical consensus splicing donor and acceptor sites in the 214-base-pair intron separating the L1 from the L2–V gene segments, and both contain an apparent TATA box 53 base pairs upstream of the translation start site. Parslow *et al.* (35) and Falkner and Zachau (27) have demonstrated the existence of a conserved decamer (*dc*) ≈100 base pairs upstream of immunoglobulin L- and H-chain V region genes. The importance of this region for expression of κ genes transfected into lymphoid cells of the B lineage has been demonstrated (36, 37), and there is evidence for protein factors that bind to this region of DNA (38, 39). The octanucleotide sequence, *ctttgcat*, present from nucleotides −101 to −92 5′ to the C.C58 gene (Fig. 3) is identical to the octamer sequence of other functional κ-chain *V* genes (27). The substitution in the BALB/c sequence of *g* for *a* (Fig. 3, position −94) to give the octamer, *ctttgcgt*, has not been previously observed and it might reduce the frequency of expression of the BALB/c $V_\kappa Ser$ gene by, for example, weakening the binding of a protein factor necessary for expression. We have conducted experiments in which a 160-base-pair *Sac* I/*Pvu* II fragment containing the conserved *dc* sequence of the functional C.C58 M75 myeloma κ-chain gene has been replaced by the corresponding fragment of the BALB/c $V_\kappa Ser$ gene, which contains the atypical octamer sequence and two additional single base-pair substitutions (Fig. 3). Both genes have been cloned into the pSV2gpt vector (40), and comparison of gene transcription after transfection into suitable target cells should determine the effect, if any, of these nucleotide substitutions.

Divergence of Upstream Sequences. As shown in Fig. 1, the restriction maps of the C.C58 and BALB/c V_κSer genomic clones diverge extensively ≈0.65 kilobases upstream of the coding sequences. As shown in Fig. 3, the absence from BALB/c of a *Hin*dIII site present at position −650 in C.C58 is due to a 2-base-pair insertion within what would be the *Hin*dIII site. The loss of this *Hin*dIII site in BALB/c is the basis for the V_κSer *Hin*dIII restriction polymorphism observed between C.C58 and BALB/c (13). A high degree of homology between the two genes exists at least 284 base pairs further upstream. A segment of DNA containing 12 copies of the 4-base repeat, *tgta*, and one copy of *ggta* is observed from position −712 to −763 of C.C58, and the comparable position of BALB/c contains only 9 *tgta* repeats. The significance, if

any, of this repetitive sequence and of the difference in its length in the two strains is not known.

While the exact position at which the nucleotide sequences upstream of the C.C58 and BALB/c $V_\kappa Ser$ genes diverge significantly has not been determined, evidence strongly suggests the insertion of a repetitive DNA element upstream of the C.C58 gene. The nucleotide sequence of the ≈500-base-pair *Bam*HI/*Bam*HI fragment, which lies upstream of the C.C58 $V_\kappa Ser$ gene (Fig. 1), is homologous to the Bam5 DNA fragment, which is a constituent of the complex L1 repetitive DNA unit (41, 42) and is present in $\approx 1 \times 10^5$ copies in the mouse genome. The nucleotide sequence immediately downstream of this element and extending to the nearby *Eco*RI site demonstrates homology with the R repetitive DNA unit (43, 44), which is also a constituent of the larger L1 repetitive unit (45, 46). Finally, sequence homology between the C.C58 and BALB/c sequences is again observed a short distance upstream of the putative C.C58 insertion (data not shown). Thus, the major difference in the restriction maps immediately upstream of the C.C58 and BALB/c $V_\kappa Ser$ genes appears to reflect the insertion of a Bam5/R composite repetitive unit upstream of the C.C58 gene. This insertion introduces one *Eco*RI site and two *Bam*HI sites upstream of the C.C58 $V_\kappa Ser$ gene that are not present upstream of the BALB/c gene. These differences are responsible for the *Eco*RI and *Bam*HI restriction polymorphisms described by Goldrick *et al.* (13) using a V_κSer-specific probe. Wilson and Storb (47) and others have previously observed the presence of Bam5/R composite elements in the vicinity of immunoglobulin *V* genes. It is possible that other *Eco*RI and *Bam*HI restriction enzyme polymorphisms associated with V_κ genes (14, 15) are also due to insertion of repetitive elements of this type.

It has been suggested that repetitive DNA may affect the expression of neighboring genes (48, 49). It is not known whether the difference in distribution of repetitive DNA in the neighborhood of the C.C58 and BALB/c $V_\kappa Ser$ genes has any effect on expression of the two genes. It is possible, however, that the divergence observed between the two genes, which by their homology are almost certainly allelic, has been in some way fostered by the introduction of repetitive DNA elements into flanking DNA regions, and that insertion of repetitive elements of this type may have been a general force in the divergence of V_κ genes among mice. We have suggested previously on the basis of restriction enzyme polymorphisms that the *Igk-VSer*^{a} allele of the inbred C.C58 strain is derived from Asian mice as seen in the recently inbred wild mouse strains MOLF/Ei and CAST/Ei (21). The *Igk-VSer*^{b} allele of BALB/c and most other inbred strains is likely to have originated in mice of European or Middle Eastern ancestry as seen in the IS/CamEi strain. We have defined two additional $V_\kappa Ser$ alleles, *Igk-VSer*^{c} and *Igk-VSer*^{d}, in other recently inbred wild mice from different geographical locations on the basis of *Eco*RI and *Bam*HI restriction enzyme polymorphisms (21). Analysis of these alleles for comparison with C.C58 and BALB/c should shed further light on the nature and mechanisms of divergence of the *Igk-VSer* locus of mice. Findings may have relevance for the evolution and divergence of other gene families at the V_κ locus and perhaps for other multigene families as well.

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1. Tonegawa, S. (1983) *Nature (London)* **302**, 575–581.
2. Weigert, M., Potter, M. & Sachs, D. (1975) *Immunogenetics* **1**, 511–523.
3. Siekevitz, M., Huang, S. Y. & Gefter, M. (1983) *Eur. J. Immunol.* **13**, 123–132.
4. Bothwell, A. L. M., Paskind, M., Reth, M., Imanishi-Kari, T., Rajewsky, K. & Baltimore, D. (1981) *Cell* **24**, 625–637.
5. Loh, D. Y., Bothwell, A. L. M., White-Scharf, M. E., Imanishi-Kari, T. & Baltimore, D. (1983) *Cell* **33**, 85–93.
6. Gottlieb, P. D. (1974) *J. Exp. Med.* **140**, 1432–1437.
7. Gibson, D. M., Taylor, B. A. & Cherry, M. (1978) *J. Immunol.* **121**, 1585–1590.
8. Laskin, J. A., Gray, A., Nisonoff, A., Klinman, N. & Gottlieb, P. D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4600–4604.
9. Dzierzak, E. A., Janeway, C. A., Rosenstein, R. W. & Gottlieb, P. D. (1980) *J. Exp. Med.* **152**, 720–729.
10. Claflin, J. L. (1976) *Eur. J. Immunol.* **6**, 666–668.
11. Gibson, D. & MacLean, S. J. (1979) *J. Exp. Med.* **149**, 1477–1486.
12. Lazure, C., Hum, W.-T. & Gibson, D. M. (1980) *J. Exp. Med.* **152**, 555–564.
13. Goldrick, M. M., Boyd, R. T., Ponath, P. D., Lou, S.-Y. & Gottlieb, P. D. (1985) *J. Exp. Med.* **162**, 713–728.
14. Huppi, K., Jouvin-Marche, E., Scott, C., Potter, M. & Weigert, M. (1985) *Immunogenetics* **21**, 445–457.
15. Moynet, D., MacLean, S. J., Ng, K. H., Anctil, D. & Gibson, D. M. (1985) *J. Immunol.* **135**, 727–732.
16. Edelman, G. M. & Gottlieb, P. D. (1970) *Proc. Natl. Acad. Sci. USA* **67**, 1192–1199.
17. Gibson, D. (1976) *J. Exp. Med.* **144**, 298–303.
18. Gottlieb, P. D., Tsang, H. C.-W., Gibson, D. M. & Cannon, L. E. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 559–563.
19. Swan, D., D'Eustachio, P., Leinwand, L., Seidman, J., Keithley, D. & Ruddle, F. H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2735–2739.
20. Gottlieb, P. D., Marshak-Rothstein, A., Auditore-Hargreaves, K., Berkoben, D. B., August, D. A., Rosche, R. M. & Benedetto, J. D. (1980) *Immunogenetics* **10**, 545–555.
21. Gottlieb, P. D., Boyd, R. T., Ponath, P. D. & Goldrick, M. M. (1986) *Curr. Top. Microbiol. Immunol.* **127**, 186–192.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1983) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
23. Frischauf, A.-M., Lehrach, H., Poustka, A. & Murray, N. (1983) *J. Mol. Biol.* **170**, 827–842.
24. Messing, J. (1983) *Methods Immunol.* **101**, 20–78.
25. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
26. Poncz, M., Solowiejczyk, D., Ballantine, M., Schwartz, E. & Surrey, S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4298–4302.
27. Falkner, F. G. & Zachau, H. G. (1984) *Nature (London)* **310**, 71–74.
28. Perlmutter, R. M., Berson, B., Griffin, J. & Hood, L. (1985) *J. Exp. Med.* **162**, 1998–2016.
29. Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M. & Perry, H. (1983) *Sequences of Proteins of Immunological Interest* (U.S. Public Health Service, National Institutes of Health, Bethesda, MD), pp. 45–64.
30. IUPAC-IUB Commission on Biochemical Nomenclature (1968) *J. Biol. Chem.* **243**, 3557–3559.
31. Nishi, M., Kataoka, T. & Honjo, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6399–6403.
32. Loh, E., Black, B., Riblet, R., Weigert, M., Hood, J. M. & Hood, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1395–1399.
33. Potter, M., Newell, J. B., Rudikoff, S. & Haber, E. (1982) *Mol. Immunol.* **19**, 1619–1630.
34. Manser, T. & Gefter, M. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2470–2474.
35. Parslow, T. G., Blair, P. L., Murphy, W. J. & Granner, P. K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2650–2654.
36. Bergman, Y., Rice, D., Grosschedl, R. & Baltimore, D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7041–7045.
37. Foster, J., Stafford, J. & Queen, C. (1985) *Nature (London)* **319**, 423–425.
38. Singh, H., Sen, R., Baltimore, D. & Sharp, P. A. (1986) *Nature (London)* **319**, 154–158.
39. Staudt, L. M., Singh, H., Sharp, P. A. & Baltimore, D. (1986) *J. Cell. Biochem.* **10**, Suppl. 10D, 191.
40. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2072–2076.
41. Fanning, T. G. (1982) *Nucleic Acids Res.* **10**, 5003–5012.
42. Rogers, J. (1983) *Nature (London)* **306**, 113–114.
43. Gebhard, W., Meitinger, T., Hochtl, J. & Zachau, H. G. (1982) *J. Mol. Biol.* **157**, 453–471.
44. Meunier-Rotival, M., Soriano, P., Cuny, G., Strauss, F. & Bernardi, G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 355–359.
45. Voliva, C. F., Martin, S. L., Hutchinson, C. A., III, & Edgell, M. H. (1984) *J. Mol. Biol.* **178**, 795–813.
46. Martin, S. L., Voliva, C. F., Burton, F. H., Edgell, M. H. & Hutchinson, C. A., III (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2308–2312.
47. Wilson, R. & Storb, U. (1983) *Nucleic Acids Res.* **11**, 1803–1817.
48. Economov-Pachnis, A., Lohse, M. A., Furano, A. V. & Tsichlis, P. N. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2857–2861.
49. Hardies, S. C., Martin, S. L., Voliva, C. F., Hutchinson, C. A., III, & Edgell, M. H. (1986) *Mol. Biol. Evol.* **3**, 109–125.