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Yeast artificial chromosome contigs reveal that distal variable-region genes reside at least 3 megabases from the joining regions in the murine immunoglobulin κ locus

(physical mapping/genomic walking/PCR screening)

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ABSTRACT **The immunoglobulin κ gene locus encodes 95% of the light chains of murine antibody molecules and is thought to contain up to 300 variable (V_κ)-region genes generally considered to comprise 20 families. To delineate the locus we have isolated 29 yeast artificial chromosome genomic clones that form two contigs, span >3.5 megabases, and contain two known non-immunoglobulin κ markers. Using PCR primers specific for 19 V_κ gene families and Southern analysis, we have refined the genetically defined order of these V_κ gene families. Of these, $V_\kappa 2$ maps at least 3.0 Mb from the joining (J_κ) region and appears to be the most distal V_κ gene segment.**

Delineation of the cis-acting regulatory elements and their interacting trans-acting factors within the immunoglobulin (Ig) genes has provided a paradigm for understanding the mechanisms of tissue-specific gene expression (1). The production of functional antibody molecules occurs only in B lymphocytes (2), requires site-specific recombination between variable (V)- and joining (J)-region gene segments immediately upstream of a constant (C)-region exon (3), and is augmented by somatic hypermutation during affinity maturation (4). The molecular bases for these intriguing biological phenomena are still not fully understood. It is likely that the complete solution to these and related questions will require the isolation of entire antibody gene clusters from the genome.

Much progress has been made in a structural analysis of the Ig κ light-chain gene locus in the human. The locus consists of two repeated V_κ gene clusters along with a single J_κ–C_κ region, spanning >3 megabases (Mb) (5). However, until now, structural analysis of the corresponding region of the mouse has only been fragmentary (see below). The experimental tractability of the mouse system has provided the impetus for the present study of developing a contig of the murine κ locus.

The Ig κ locus in the mouse contributes 95% of the light chains of antibody molecules. It contains 1 C_κ exon, 5 J_κ segments (4 of which are functional) and up to 300 V_κ gene segments, although probably less (6). The physical extent of the locus is unknown but has been estimated to range from 500 to 2000 kb (7). The V_κ gene segments have been organized into 20 families based on nucleotide sequence homology (8–11). A single V_κ gene family ranges in size from 1 to >30 members with each sharing >80% sequence similarity (8) and is characterized by a discrete restriction fragment length polymorphism (RFLP) hybridization pattern (6, 12). Analysis of these RFLP patterns in inbred strains of mice has been used to generate a genetic map of the Ig κ locus (6, 12), as well as to map Ig κ relative to other genetic markers (13–16). The $V_\kappa 21$ gene family has 6 members that have been physically linked (17). Members of the $V_\kappa 1$ and $V_\kappa 9$ families have also been physically linked (18). Other than these examples, a number of germline clones of various V_κ genes have been isolated but have not been linked (8).

In this report we present two yeast artificial chromosome (YAC) contigs that encompass 19 mouse V_κ gene families and span >3.5 Mb. Using V_κ family-specific PCR, we have been able to align V_κ gene families in a manner consistent with published genetic data and to confirm the alignment by physical mapping. We have identified the putative 5′ end of the locus, including distal V_κ gene families. These studies are an essential prerequisite so that Ig κ regulation may be fully understood.

MATERIALS AND METHODS

Library Screening. The Princeton Mouse YAC Complex pool (19) and a large-insert library (20) were screened with J_κ–C_κ, $V_\kappa 24$, and $V_\kappa 28$ primers. The large-insert library was further screened with $V_\kappa 2$, $V_\kappa 10$ and $V_\kappa 12$ primers. Other V_κ family primers were used for further characterization (see below). PCR mixtures contained 50 mM KCl; 10 mM Tris Cl (pH 8.3); 1.5 mM $MgCl_2$; 200 μM each dATP, dCTP, dGTP, and dTTP; 0.001% Tween-20; 0.001% Nonidet P-40; 50 μM each primer; and 2 units of *Taq* DNA polymerase in 100 μl and were overlaid with 50 μl of light mineral oil. Reactions were 1 min at 92°C, 2 min at 55°C, and 1 min at 72°C for 50 cycles. The final cycle was extended for 10 min. Ten microliters of each PCR mixture was analyzed by gel electrophoresis and subsequent Southern blotting. For PCR analysis of isolated clones, the number of cycles was reduced to 30.

PCR Primers. Nucleotide sequences (5′ to 3′) of forward and reverse (respectively) primer pairs were as follows: J_κ–C_κ intron, CAGGTAGCGTGGTCTTCTAGACG, CTTGCAGCTGTCTGTCAAGGG; $V_\kappa 2$, GCCCAGTTCCTGTTTCTGTTAGTGC, TCCAGAGTCCAGTTTAGACACCAGA; $V_\kappa 10$, AGGGCAAGTCAGGACATTAGCAATTAT, GGAAGCGTATTACCCTGTTGGCAAA; $V_\kappa 24$, AAAAGCTAGCCCTCTTCCTTCCTCG, GAGGATACTCTACAAGTTGTTGACAG; $V_\kappa 28$, ATGAAGTCACAGACCCAGGTCTTC, GGAGAGCTATAATCCTGCTGACAG; $V_\kappa 12$, CTGGCGTTGCTGCTGCTGTGGC, AGGAGTACTCCAAAAATGTTGACTG; $V_\kappa 1$, TTGCCTGTTAGGCTGTTGGTGCTG, CCCAGAAAATCGGTTGGAAACTTTG; $V_\kappa 4$, CARGTGCAGATTTTCACTTCCTGC, ACTCCAGAAGCCAGGTTGGATGTG; $V_\kappa Ox$, GATTTTCAGCTTCCTGCTAATCAGTGC, GGTAACCACTGTACTGCTGGCAG; $V_\kappa 8$, TGTTCTGGGTATCTGGTACCTGTG, CACTGTTTAACAGACTCTGACTGGAC; $V_\kappa 9$, CAGAT-

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Abbreviations: FISH, fluorescence *in situ* hybridization; RFLP, restriction fragment length polymorphism; YAC, yeast artificial chromosome.

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TTTTGGCTTCTTGTTGCTCTTG, CCAGTTTAAGCTACTACCAATGTCC; V_κ11, AGAACCCTGCTCCTTTCCTTGGGC, CCCCATCTTCCAATTTTGTTTGCACC; V_κ19, GCCAGTCAGAATGTTCGTACTGCT, GATAATTCCAATGTTGCAGACAGAAA; V_κ20, GCCTTCTTCTCCTCTGTGTCTCTG, GGCTAGTTATCACTTTGCAAACAGTAG; V_κ21, TGCTGCTGCTCTGGGTTCCAGGTG, GATTCTAGGTTGGATGCAGSATA; V_κ22, CTATTTCTTATTGTAGGTGCCTCGTG, GCTGTAAAACTGTTGCACAGTAATAATG; V_κ23, GCCAGCCAGAGTATTAGCGACTACT, CGGAGGAAAGCTGTGACCATTTTGAC; V_κ32, AGGGTCCTTGCTGAGCTCCTGG, GGCTAGTTATCACTTTGCAAACAGTAG; V_κ33, AAGTTTCCTTCTCAACTTCTGCTCTTA, TCCGATATATATGTCCTCAGTTGCC; V_κ34, TCCTTTTCAACTTCTGCTCTTCCTGC, CGGAGGAGTACTCCAATACTGTTG.

Primary references for V_κ gene sequences are cited in ref. 8, with the exception of V_κ20 (10), V_κ32/V_κ33 (11), and V_κ34 (9).

End-Fragment Rescue, Electrophoresis, and Southern Analysis. Material adjacent to the left (*TRP1*) and right (*URA3*) arms of YACs was rescued by hemi-nested bubble PCR (21). Amplification conditions were 92°C for 1 min, 67°C for 2 min, and 72°C for 2 min for 20 cycles. Vector material was removed from probes by *Eco*RI digestion and gel purification. Bubble probes were annealed with 50 μg of Cot-1 DNA (GIBCO/BRL) for 1 hr prior to hybridization. DNA was purified from yeast cells embedded in 0.75% InCert agarose (FMC BioProducts). For mapping, YACs were subjected to partial restriction digestion and separated by pulsed-field gel electrophoresis in a CHEF Mapper XA (Bio-Rad) for indirect end-labeling with vector arm probes after Southern transfer. Typical run conditions were 1% FastLane agarose (FMC BioProducts), 0.25 × TBE (1× TBE is 90 mM Tris/90 mM boric acid/2 mM EDTA), 6 V/cm for 15 hr with a 60-s switch time, followed by 9 hr with a 90-s switch time. Prior to transfer, DNA was nicked by exposure to UV light (150 mJ). Standard techniques were used for the resolution of DNA fragments <20 kb in size (22). Probes were labeled with [α-^{32}P]dCTP (Amersham) by the Megaprime labeling system (Amersham). Filters were hybridized as described (23). The Rn7s (14) and Mtv-8 (13) probes were gifts of B. Taylor and J. Dudley, respectively. Genomic DNA comparisons were made to C57BL/6J DNA purified from liver.

Fluorescence *in Situ* Hybridization (FISH). YAC DNAs (1 μg) were labeled with biotinylated-dATP by BioNick (GIBCO/BRL) and hybridized to metaphase chromosome

	2	20	1	9A	11	24	34	33	32	10	X	4	12	23	8	22	28	19	21	Ck	Size (kb)	Chi?
FDG.G10	+	+	+	-	-	-						-									750	Y*
AAH.F4	+	+	+	-	-	-						-									440	Y*
FBB.A3	+	+	-	-	-	-	-	-	-	-	-	-	-								720	N
FBW.G7	+	+	-	-	-	-	-					-									850	N
ADV.F9	+	+	+	-	-	-	-	-	-	-	-	-	-								240	Y
FDW.H8	+	+	+	-	-	-						-									560	Y
FAR.E8	+	+	+	+	+	-	-	-				-									550	N
D19.B3.4	-	+	+	+	+	+	-	-	-	-		-									310	
FDA.G2	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-				1200	N
FCK.B1	-	-	+	-	+	+	+	+	-	+	+	+	-	-	-	-	-				850	
AAH.C8	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-						480	
AAH.B5	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-				480	
FAW.F4	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-			700	N
FBZ.D4	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-				680	N
FFA.A3	-	-	-				-	-	+	+	+	-	-	-		-	-				1400	
FCH.D1	-	-	-	-	+	-		-	+	+	+	+	-	-	-						500	
FDC.H10							-	-	-	-	+	+	+	-	-	-	-	-			630	
FEA.H5							-	-		-	+	+	+	+	-	-					610	
FFN.D5							-	-		-	+	+	+	+	-	-						
ACQ.D3							-	-		-	+	+	+	+	-	-					440	N
AAN.G7							-	-		-	+	+	+	+	-	-					325	N
FDC.E4							-	-	-	-	+	+	+	+	+	+	+	-			650	N
FEH.A4							-	-		-	-	+	+	+	-	-					365	
FCE.G10	-		-		-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	640	N
C186.D11	-		-		-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	280	
C63.D7	-		-		-	-	-	-	-	-	-		-	-	+	+	+	-	-	-	450	
A18.B1	-		-		-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	350	
FAW.A3	-		-		-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	1270	Y
FBD.B9	-		-		-	-	-	-	-	-	-			-			-	-	+	+	280	

FIG. 1. Summary of Ig κ clones and their V_κ gene family content. Princeton YAC libraries were screened as described (*Materials and Methods*). Top row indicates V_κ gene family, YAC size, and chimerism (Chi?), as determined by FISH. X indicates V_κOx. The columns indicate clone name (left), the presence or absence (+ or −) of a signal by ethidium bromide staining, and chimerism (Y, yes; N, no). Nonchimeric YACs (N) hybridize only to chromosome 6. Blank indicates not done. Asterisk indicates complex hybridization pattern, not including chromosome 6. Some YACs (yFBB.A3, yFAR.E8, yFDA.G2, yFBZ.D4, yFDC.E4, and yFAW.A3) were assayed for the presence of markers known to map near the Ig κ locus, including *D6Mit16, D6Mit17, D6Mit19, D6Mit71, D6Mit72, D6Mit124* (ref. 25; Massachusetts Institute of Technology database]. None of these markers were detected within the YACs. The Ig κ-specific marker *D6Mit96* was detected in yFDC.E4. *D6Mit97*, also Ig κ-specific, was detected in yFAR.E8 and yFDA.G2.

spreads prepared from bone marrow cells of mice bearing a Robertsonian translocation between chromosome 6 and 18 [*Rb*(6.18)*Dn*; The Jackson Laboratory]. Detection was performed as described (24) with avidin–fluorescein isothiocyanate conjugate and biotinylated anti-avidin from Vector Laboratories.

RESULTS AND DISCUSSION

YAC Clones Containing the Ig κ Locus. Twenty-nine YACs containing Ig κ sequences were isolated that ranged in size from 240 kb to 1400 kb (Fig. 1). An extended set of PCR primers was used to analyze V_κ gene content, resulting in a presumptive contig with a predicted order of V_κ gene families. The V_κ families were arrayed with the assumption that the majority of YACs were intact (Fig. 1). Where there were discrepancies in the V_κ gene order between YACs, the order assigned exhibited the fewest inconsistencies. Regardless, there are some positions within the PCR array of YACs that are consistent with some YACs having deletions (Fig. 1). Interspersed V_κ family members or crossreactive PCR priming are other explanations for inconsistencies. To check the fidelity of PCRs, we cloned and sequenced 60 products from 10 different V-region families. In all cases examined ($V_\kappa1$, $V_\kappa2$, $V_\kappa4$, $V_\kappa Ox$, $V_\kappa11$, $V_\kappa12$, $V_\kappa20$, $V_\kappa21$, $V_\kappa19$, and $V_\kappa23$), all cloned PCR products corresponded to the appropriate V_κ gene family (data not shown). However, there is also the possibility that, for certain V_κ gene families, primer sequence mismatches may not allow detection of all family members. The data in Fig. 1 provided a useful starting point for further analysis.

Physical Map of the Ig κ Locus. To confirm the contig predicted by the PCR analysis and orient the YACs with respect to each other, insert DNA was rescued from both ends of 16 YACs. Reciprocal hybridization of identical restriction fragments between two YACs was used to demonstrate overlap. Chimeric YACs, determined by FISH or inferred (i.e., yFFA.A3), were eliminated from the analysis. Exhaustive combinations of probes to YACs revealed two nonoverlapping contigs accounting for 3.5 Mb. The two contigs were further verified by restriction mapping as shown in Fig. 2. Also indicated in Fig. 2 are the positions of fragments hybridizing to $V_\kappa2$, $V_\kappa20$, $V_\kappa4$, $V_\kappa21$, and C_κ probes, as well as two non-Ig κ sequences, Mtv-8 and Rn7s. Comparisons with genomic DNA show that these non-Ig κ regions are intact within our YACs (data not shown). As anticipated, Mtv-8 maps in a YAC that contains $V_\kappa9$ (13), and Rn7s maps near $V_\kappa28$ (14) (Fig. 2; see also Fig. 5). yACQ.D3 is the only YAC that contains the Rn7s marker (Fig. 2), supporting the observation of a gap. The gap between these contigs presents a potential problem in determining the relative polarity of one contig with respect to the other. The orientation presented is inferred by the strong concordance with genetic mapping data (see below).

Southern Analysis of the Gap Between Contigs. The region that fails to overlap contains the most complex V_κ gene segment, $V_\kappa4$, which comprises as many as 50 members and includes $V_\kappa Ox$ as a subset (26). By comparison of V_κ hybridization patterns of various YACs and mouse genomic DNA, we observe that the majority of $V_\kappa4$ family members are present in the two contigs, but at least 2 members are not accounted for (Fig. 3*a*, arrowheads). Based on previous V_κ spacing estimates (17) and assuming that the missing $V_\kappa4$ bands are within the gap, we anticipate the size of this gap to be at least 30 kb. In contrast, when we compare the hybridization patterns of $V_\kappa10$ and $V_\kappa12$ probes between the YACs and genomic DNA, all the members are accounted for and present on the 5′ or 3′ side of the gap, respectively (Fig. 3 *b* and *c*).

Distal V_κ Gene Families. PCR-based mapping data suggested that either $V_\kappa2$ or $V_\kappa20$ is the terminal V_κ gene family (Fig. 1). Southern analysis of the mapped YACs reveals that fragments hybridizing to a $V_\kappa2$ probe reside only in the two 5′-most YACs (Fig. 2) and that all $V_\kappa2$-positive fragments are shared between these two YACs (Fig. 4). Thus, although yFBB.A3 extends 570 kb farther 5′ than yFAR.E8, it does not appear to contain any other $V_\kappa2$ gene segments. Hybridization of the same filter with a $V_\kappa20$ probe likewise indicates that no members of $V_\kappa20$ are present in yFBB.A3 that are not represented in yFAR.E8. In addition, two other $V_\kappa20$ members are shared between yFAR.E8 and yFDA.G2. PFGE mapping places $V_\kappa2$ and $V_\kappa20$ in a 150-kb region of overlap between yFBB.A3 and yFAR.E8, with $V_\kappa20$ mapping only to the 3′-terminal 80 kb of yFBB.A3 (Fig. 2) as well as in yFAR.E8 and yFDA.G2. These data suggest that $V_\kappa2$ is the most-5′ known V_κ gene segment. Genomic Southern blots probed for either $V_\kappa2$ or $V_\kappa20$ contain bands (one or two, respectively) not found in any of the mapped YACs. Hybridization to the other $V_\kappa2$-containing YACs reveals that all of these fragments are accounted for in each of two YACs, yAAH.F4 and yFDG.G10. Upon FISH analysis, both of these YACs exhibit complex hybridization patterns, indicating that they are chimeric. Significantly, neither YAC hybridizes to chromosome 6. These observations are consistent with yFDG.G10 and yAAH.F4

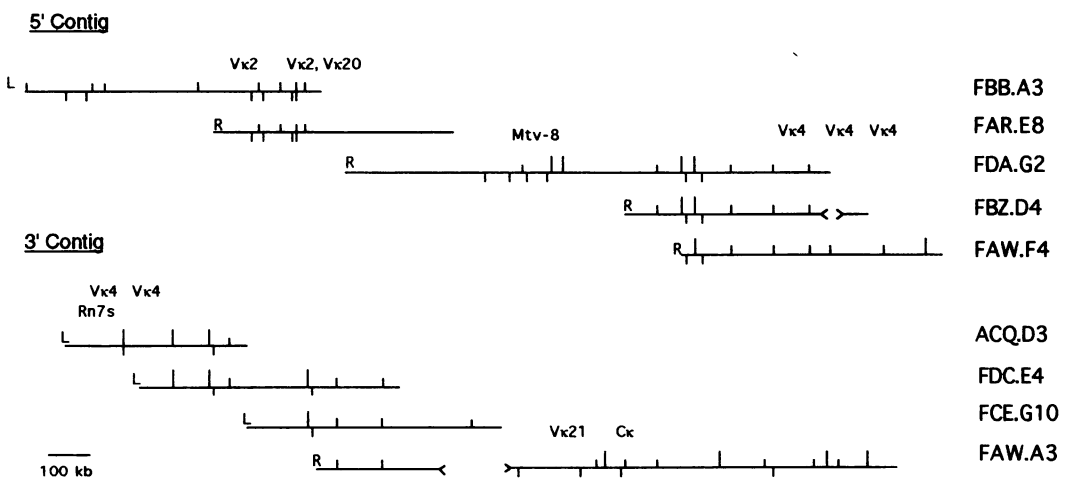

FIG. 2. Restriction map of YAC contigs spanning the Ig κ locus. A subset of clones were selected for generating a contig and mapping. All clones shown appear nonchimeric by both FISH analysis and the ability of the end-rescued probes to hybridize to identical restriction fragments of adjacent clones, with the exceptions of yFAW.A3 and FCE.G10 (FISH only). ⊥, *Bss*HII; ⊤, *Ksp* I; ⊥, *Eag* I; – – –, chimeric material; <>, deletion; L and R, left and right vector arms, respectively.

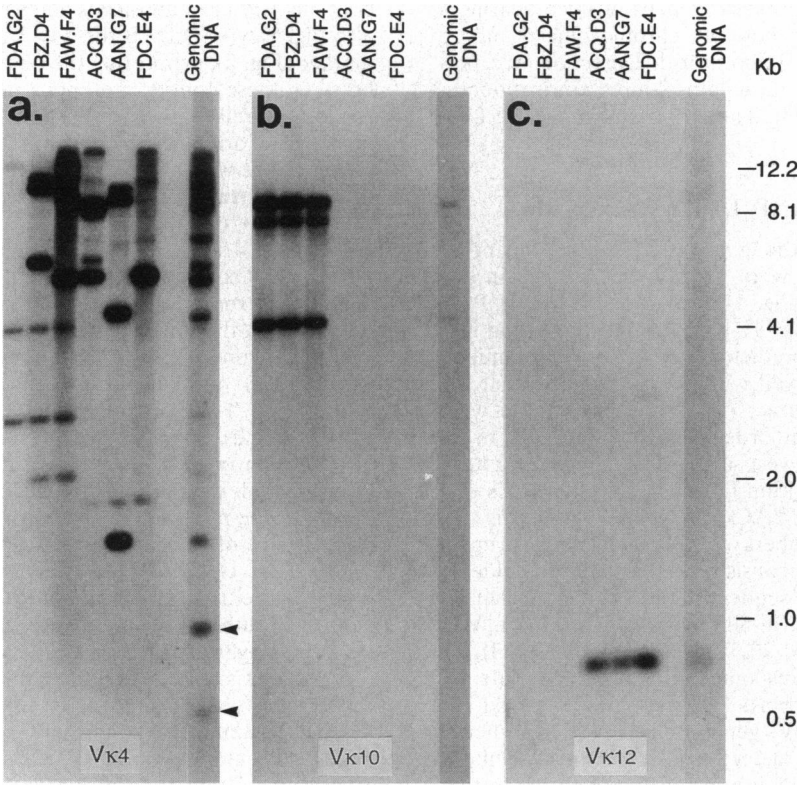

FIG. 3. Examination of the gap between YAC contigs by Southern hybridization analysis of *Pst* I-digested YACs and mouse genomic DNA with the indicated V_κ probes, PCR-amplified from mouse genomic DNA. Arrowheads indicate the bands present only in mouse genomic DNA.

harboring an "orphon" cluster of V_κ gene segments similar to those seen in the human (27).

Comparison of Genetic and Physical Maps. As summarized in Fig. 5, the V_κ gene order described by our PCR analysis is highly consistent with the genetic maps generated by RFLP analyses and consistent with the assumption that V_κ families are generally clustered. The differences we observe presumably result from technical differences between the two mapping strategies, such as RFLP mapping being contingent on detection of a polymorphism. There are four exceptions of note. First is the position of the Rn7s marker, which is found only near the left end of yACQ.D3 (Fig. 2). While our data (PCR, Southern hybridization, and restriction mapping) confirm the linkage between Rn7s and V_κ28, Rn7s appears farther from V_κ28 than had been anticipated, with several V_κ gene families intervening. Second is the position of V_κ23. In the genetic studies, V_κ23 was linked with V_κ4, V_κ8, V_κ10, V_κ12, V_κ19, and V_κ28 (as well as Rn7s) as a group in the wild-type strains analyzed (7). Analysis of congenic mice indicated a breakpoint between Rn7s and V_κ23, with both of these markers lying 3′ of V_κ28. Our study supports this linkage, but at a position 5′ of V_κ28. In those YACs where we have looked, the V_κ23 RFLP is intact (data not shown). Third, V_κ32 has been predicted to be a dispersed gene family, mapping in two positions, first in the group of V_κ gene families that includes V_κ1, V_κ9, V_κ11, and V_κ24, and second, mapping near V_κ28 (17, 26). Our data suggest that only the former is accurate. Finally, a discrepancy is noted for the placement of V_κ33, possibly due to the fact that the genetic mapping data for V_κ33 are scant, based on a single RFLP observed between two strains of mice (11). Despite the discordance of V_κ32, V_κ33, and V_κ34 with other V_κ families, these three families map near each other in both the genetic studies and in the work presented here.

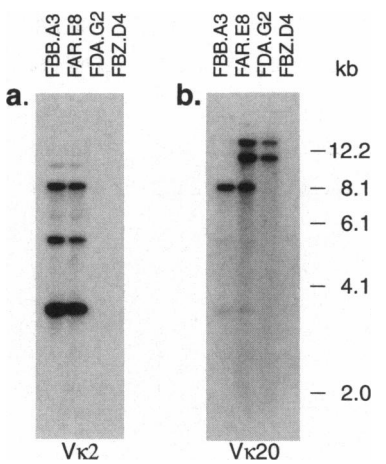

FIG. 4. Distal V_κ gene families. *Eco*RI-digested YAC DNAs were hybridized to V_κ2 (*a*) and V_κ20 (*b*) probes, PCR-amplified from mouse genomic DNA.

Conclusions and Prospectus. In summary, we have isolated the majority of the murine Ig κ locus on a series of YACs. Our data indicate that the Ig κ locus spans >3.0 Mb. We present a refined order for 19 V_κ gene families, identify the putative 5′ end of the locus, and show the positions of two independent non-Ig κ markers.

The fact that the Ig κ locus spans at least 3.0 Mb of the genome raises interesting questions about how the locus is activated both during ontogeny and during ongoing lymphopoeisis in adult animals. To date, aside from V-region promoters, all characterized regulatory elements—including sterile promoters, two enhancers, and a nuclear matrix association region (MAR) together span only 20 kb of the locus and all reside at the 3′ end, near the J_κ–C_κ region (2, 29). Transgenic experiments with a human germline construct containing all the corresponding elements has demonstrated relatively poor expression (30, 31). This observation suggests that other regulatory elements may exist, perhaps in the far upstream region, which could play a role in propagating activation signals

a. (11, 24, 9-26)- (1, 9)- (8, 4/5, 10, 12, 19)- (28, Rn7s)- 23- 21- Cκ
b. (1, 2, 9A, 11, 20, 24, 32)- (4/5, 8, 10, 12, 22, 19/28)- (19/28, 32, 33/34)- 23- 21- Cκ
c. 2- 20- 1- 9A- 11- 24- 34- 33- 32- 10- (4/5- Rn7s -12- 23)- 8- 22- 28- 19 - 21- Cκ

FIG. 5. Comparison of genetic and PCR V_κ family mapping data. Order of V_κ gene families is presented from ref. 7 (*a*), ref. 28 (*b*), and our PCR-based analysis of YACs (*c*). The order of V_κ families within a single bracket cannot be resolved. Underline indicates V_κ families whose position within a contig is consistent with genetic data.

across 3.0 Mb of the genome. Subsequent to global activation and rearrangement, remaining V_κ genes that have not undergone successful rearrangement are returned to an inactive state. We do not know what elements are involved in shutting down sterile transcription (3) and early replication (32) from these regions. Moreover, observation of such a large, tissue-specifically regulated domain raises questions as to whether and how higher-order chromatin structure and nuclear packaging might determine gene expression. Because of the exceptional size of the Ig κ locus, YAC clones spanning this region provide a powerful tool for investigating these and related questions.

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