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Physical Linkage of Mouse λ Genes by Pulsed-Field Gel Electrophoresis Suggests that the Rearrangement Process Favors Proximate Target Sequences

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The first complete map of a mammalian immunoglobulin gene locus is presented. Mouse λ genes were mapped by pulsed-field gel electrophoresis. The gene order is V2-Vx-C2-C4-V1-C3-C1. The distance between V2 or Vx and the C2-C4 cluster is 74 or 55 kilobases (kb), respectively, whereas that between V1 and C3-C1 is only 19 kb; V2 and C3-C1 are at least 190 kb apart. Thus, the distances between the λ subloci are inversely proportional to their frequencies of rearrangement. The related gene λ5 is not within the 500 kb of the λ locus mapped here.

Mouse λ genes have been found in four separate clusters of DNA clones which so far were physically unlinked: a cluster of clones containing the JC3 and JC1 genes, one of JC2 and the pseudogene JC4, and separate groups of clones for V1 and V2 (2, 31). Interestingly, the lambda genes rearrange in restricted combinations, Vλ1 only with Cλ1 or Cλ3 and Vλ2 almost exclusively with Cλ2 but very rarely with Cλ1 or Cλ3 (7). The newly discovered Vλx gene has so far been seen rearranged only with Cλ2 (6, 28). Recently, the order and orientation of λ genes, except for Vx, were determined by deletion mapping with unique probes (18). The genes were all found to be in the same transcriptional orientation, and the order was determined to be V2-C2-C4-V1-C3-C1 (18). This organization explains why V1-C2 rearrangements were not found but gives no clues about why rearrangements of V1 are about 5 to 10 times more frequent than rearrangements of V2. Furthermore, the location of Vx in this scheme was unknown. Since the sequence of Vx is as related to Vκ as to Vλ1-Vλ2 (6), its evolutionary association with the λ locus presumably occurred in a way different from that of V1-V2 and thus its location and orientation may be unusual.

We have cloned, in phage and cosmids, approximately 140 kilobases (kb) of DNA making up the Vλ and Cλ genes and their flanking regions. However, it was impossible to link up the λ genes by chromosomal walking because of the paucity of unique sequences in this locus (18). We therefore used pulsed-field gel electrophoresis (PFGE) (3, 30, 33) in combination with unique probes for λ genes to produce a complete linkage map of λ genes.

MATERIALS AND METHODS

PFGE. DNA for PFGE was prepared by making nuclei (34) and mixing them with low-gelling-temperature agarose (SeaPlaque; FMC Corp., Marine Colloids Div., Rockland, Maine) to a final concentration of 0.6%. Forty microliters each of the mixture containing 8×10^5 cells (4.8 μg of DNA in the case of diploid cells) was pipetted into casting molds (30); the inserts were refrigerated for 30 min and then transferred into lysis buffer (0.5 M EDTA, pH 9.5, 1% *N*-lauryl sarcosine, 0.25 mg of proteinase K per ml). The agarose inserts were incubated for 48 h at 50°C, washed extensively for 48 h in 10 mM Tris (pH 7.2)–1 mM EDTA (TE) at 4°C, and stored at 4°C in TE. DNA inserts were digested in the restriction enzyme buffer specified by the manufacturer (New England BioLabs, Inc.) containing 4 mM spermidine. Each 40-μl insert was incubated in 40 μl of 2× reaction mixture with 60 U of enzyme at 37°C overnight; on the next morning, a further 40 U of enzyme, 4 μmol of spermidine, and sufficient 10× buffer and bovine serum albumin to bring these reagents to 1× were added. Incubation was continued for 4 to 6 h at 37°C, and the inserts were rinsed with 3 ml of electrophoresis buffer (0.25× TAE [16]) for 30 min on ice and electrophoresed as indicated in the figure legends.

5-Azacytidine treatment. 5-Azacytidine treatment was for 32 h in culture (9, 12); cells were then washed once and cultured for an additional 60 h in regular medium.

Hybridization probes. The hybridization probes used have been previously described (18, 28). Briefly, V2 cross-hybridizes with V1 because of 97% homology (1, 35); C2 cross-hybridizes with C3 because of 97% homology; C1 hybridizes mainly with C1 but more weakly with C4 because of 87% homology; 3′V2, 5′C2, and 3′C1 are unique probes isolated from the λ clones (see Fig. 5); and Vx was isolated from a VxC2 cDNA clone (28).

RESULTS

Linking λ genes by PFGE. In experiments designed to confirm the order of the λ genes and to determine the distances between the subloci, the PFGE patterns of the DNAs of BALB/c kidney cells and BALB/c J558L myeloma cells (20) were compared. Since J558L has both V1 genes rearranged, this comparison aids in determining the distance between V1 and C3-C1. Various restriction enzymes which cut rarely were used. The enzymes *Not*I, *Mlu*I, *Bss*HII, *Fsp*I, and *Nru*I did not yield bands which could be resolved within 800 kb on a PFGE blot. The only enzymes which resulted in distinct bands were *Xho*I, *Sal*I, and *Cla*I. The former two were used for further analysis, because several

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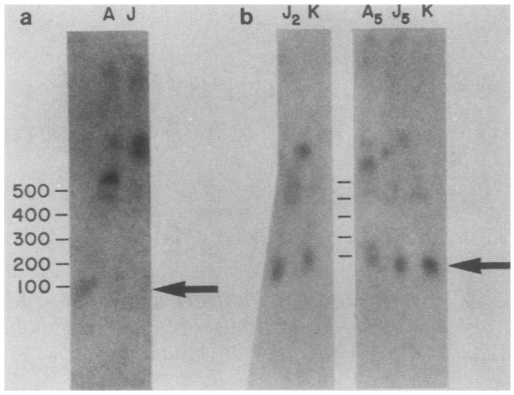

FIG. 1. Hypermethylated λ genes in a λ-producing myeloma. DNA of the myeloma Ag8.653 (A), J558L (J), or BALB/C kidney (K) was restricted with *Sal*I and submitted to PFGE on a homemade apparatus designed as described by Carle and Olson (3) at a 25-V/cm constant voltage for 15 h with a pulse of 40 s at 13°C. Hybridization of the blotted DNA was with a 3′V2 probe (18) (see Fig. 5). J2 and J5, J558L cells treated with 2 or 5 μM 5-azacytidine (9, 12). A5, Ag8.653 cells treated with 5 μM 5-azacytidine. The numbers indicate kilobases as determined by ladders of annealed λ DNA run on the same gel. The arrows show a fragment of <100 kb seen with J558L DNA only after treatment with azacytidine.

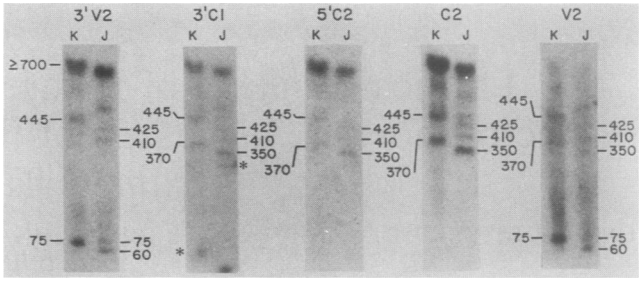

FIG. 2. PFGE of kidney and J558L DNAs digested with *Sal*I. Kidney or J558L (2 μM azacytidine treated) DNA was digested with *Sal*I and subjected to PFGE at 20-s pulses for 21 h at 16°C in 0.25× TAE (16) in a Beckman Gene Line apparatus. The DNA was blotted on Zeta-Probe (Bio-Rad) (10) and hybridized with the indicated probes. Hybridization of the four panels on the left was consecutive, as indicated, with a blot from the left part of the gel; the last panel is a blot from the right side of the same gel. Probes were labeled by random priming. Between hybridizations, the old probes were melted off and blots were exposed to X-ray film to assure complete stripping. Sizes of bands were determined from phage λ DNA ladders run as standards in the same gel. The asterisks indicate smudges on the blot, not hybridization bands. The numbers to the sides indicate molecular sizes in kilobases.

*Xho*I sites and one *Sal*I site were present in our λ clones, thus aiding the analysis.

Upon digestion with *Sal*I and probing with a sequence 3′ of V2, DNA from kidney or myeloma Ag8.653 cells (13), which has rearranged kappa genes but germ line λ genes, gave a fragment of <100 kb and two fragments in the range of 400 to 800 kb which were not seen with J558L (Fig. 1a). Likewise, with other probes (data not shown) J558L DNA always showed very large bands, suggesting that in these cells the λ locus may be hypermethylated at certain sites and therefore resistant to digestion by methylation-sensitive restriction enzymes. To prepare DNA from J558L which could be cleaved by methylation-sensitive enzymes, the cells were treated with 5-azacytidine (9, 12), an analog of cytidine which cannot be methylated, and the DNA of the treated cells was reanalyzed (Fig. 1b). Apparently, the λ locus had become at least partially unmethylated and J558L showed the diagnostic <100-kb band. For further PFGE analysis, DNA from J558L cells treated with 2 μM 5-azacytidine was used. Even after 5-azacytidine treatment of the DNA, multiple bands were obtained with 3′V2, a probe which is unique (Fig. 1) (18), i.e., hybridizes with only a single band when DNA is digested with a variety of more frequently cutting enzymes. Multiple bands were also seen with *Sal*I digestion of all of the DNAs with all of the probes, including the additional unique probes 5′C2, 3′C1, and Vx (see below). The smallest bands presumably represent complete digestion, whereas the larger bands were derived from incomplete digestion due to partial methylation at certain sites. Such partial digests were useful in determining at which fragment size multiple probes would give a common signal, suggesting the presence of the multiple sequences in a common stretch in the genome.

The data in Fig. 1 also show that the homemade flatbed apparatus for PFGE would not be useful for distinguishing band differences of less than 100 kb because of vertical and horizontal distortions and the lack of focus of the bands. We therefore switched to the recently developed Gene Line apparatus (Beckman Instruments, Inc.), which eliminated these distortions (see Fig. 2).

The hybridization probes used have been previously described (see Fig. 2 to 5) (18, 28). It must be emphasized that three of the probes cross-hybridize with two of the λ genes because of sequence homologies; V2 reacts with Vλ2 and Vλ1, and C2 reacts with Cλ2 and Cλ3. C1 hybridizes mainly with Cλ1 but also weakly with Cλ4. The C4 probe is essentially specific for Cλ4 (J. Hagman, unpublished data). Four unique probes corresponding to regions 3′ of V2, 5′ of C2, and 3′ of C1 (see triangles in Fig. 5) and Vx were used.

Digestion of kidney DNA with *Sal*I and probing with five different probes derived from sites across the λ locus showed very similar patterns for all of the probes (Fig. 2; see Table 1 for interpretation of the bands). Identical bands of 370 and 445 kb were obtained with 5′C2, C2, (C1; data not shown), and 3′C1 probes, indicating that both the C2-C4 and C3-C1 subloci are contained within 370 kb of DNA; the 445-kb band is a partial digest (see below). *Sal*I apparently has no other site within the λ locus besides the one 3′ of V2 known from the λ DNA clones (see Fig. 5). Thus, with *Sal*I, V2- and 3′V2-containing fragments could be separated from the rest of the locus. With 3′V2, only the 445-kb fragment was seen but the 370-kb band was not, indicating that the 370-kb fragment has its 5′ end at S1 and thus does not include V2 and 3′V2 (see Fig. 5 and Table 1). This defines the position of S2, 3′ of all λ genes and 370 kb 3′ of S1. The 370-kb band seen with the V2 probe must represent cross-hybridization with the V1 gene (see Fig. 5). A band of 75 kb was seen only with the V2 and 3′V2 probes. This defines a *Sal*I site (S4) upstream of V2.

Comparison of the patterns of hybridization of kidney DNA and J558L DNA showed identical bands at 75 kb with V2 and 3′V2. In addition, a 60-kb fragment was seen with J558L which was extremely faint with kidney cells (see Fig. 2 and 3 and Table 1). Apparently, site S3 was almost completely methylated in kidney cells but was largely but not completely unmethylated in J558L cells by 5-azacytidine treatment. In a comparison of the higher-molecular-weight bands, the 370-kb band was absent from J558L. Instead, a 350-kb band was seen. This suggests that the distance between S1 and S2 and thus the genomic sequence comprising most of the λ locus is about 20 kb shorter in J558L. In this

cell line, both V1 genes were rearranged to the C3-C1 cluster by deletion of the intervening DNA (18). The distance between V1 and the C3-C1 cluster therefore appeared to be about 20 kb (see Fig. 5). This conclusion is further supported by the presence of a 425-kb band of J558L DNA that hybridized with all of the probes, i.e., 20 kb shorter than the 445-kb S4-to-S2 band obtained with kidney DNA. The 410-kb fragment with J558L probably comprises S3 to S2 (see Fig. 5). The length difference between the V1-J3 and V1-J1 rearrangements of J558L should be about 4 kb, the distance between J3 and J1 (2, 31). It was apparently impossible to resolve this small difference in the PFGE blot shown. Additional larger fragments were present which presumably extend to *Sal*I sites further 5′ or 3′ (or both) of the map shown (see Fig. 5).

To obtain finer resolution within the λ locus, the DNAs were cut with *Xho*I or *Xho*I and *Sal*I and electrophoresed with a shorter pulse to achieve a greater spread between 5 and 300 kb (Fig. 3). When kidney DNA was probed with the unique 3′C1 sequence, the same fragments were obtained whether *Xho*I or *Xho*I plus *Sal*I was used (Fig. 3; see Table 1 for band interpretation), thus confirming the absence of a *Sal*I site within the 3′ part of the λ locus. The shortest fragment of 70 kb which is also positive with C1 but negative with C2 (=C3) defines X8 as the next *Xho*I site 3′ of X7. The X7 site was known on the basis of the λ clones to be located at nucleotides 54 to 59 within the C1 gene (31). The 70-kb fragment was also seen with J558L, supporting its location 3′ of the rearrangement joints of V1-J3 and V1-J1. An 80-kb fragment (which represents a partial fragment not cut at X7) positive with 3′C1, C1, and C2 (=C3) and a 9.4-kb fragment with the C2 (=C3) probe were seen only with kidney DNA, because site X6 is deleted on both homologs of chromosome 16 of J558L because of the V1-J3 and V1-J1 rearrangements (see Fig. 5).

Sites X2 and X3 are known on the basis of the λ clones to be present 5′ of C2 (see Fig. 5) (18). X2 has been placed 60 kb 3′ of X1 because the shortest *Xho*I fragment positive with 3′V2 and 5′C2 is 60 kb (Fig. 3 and 4).

The two *Xho*I sites X4 and X5 have been mapped relative to X8; with kidney DNA, two fragments of 174 and 150 kb were obtained which hybridized with 3′C1, C1, C2 (=C3), and V2 (=V1) but not with C4 (Fig. 3 and 5; Table 1). We placed X4 and X5 23 and 47 kb 3′ of X3, respectively, for the following reasons. An *Xho*I site (X4) was defined 30 and 23 kb 3′ of X2 and X3, respectively, by the 30- and 23-kb fragments which hybridize only with C4 (data not shown), C2, and C1 (=C4) (Fig. 3), the latter being a weaker signal because of only 87% homology between C1 and C4 (31). Both of these fragments were also seen with J558L, in which they were negative with the V2 (=V1) probe. Therefore, they cannot be derived from the C3-C1 region. We assumed that X4 is the same *Xho*I site which is 23 kb 3′ of X3 and 174 kb 5′ of X8, because of a faint hybridization band (with *Xho*I or *Xho*I plus *Sal*I) of about 200 kb which is positive for C4 (data not shown), V2, C2, C1, and 3′C1 but not 3′V2 (Fig. 3; Table 1). This presumably represents X3 to X8. Thus, X5, which is 24 kb 3′ of X4, comes to lie 47 kb 3′ of X3. An independent confirmation of X5 relative to X3 is a fragment of 47 kb which is positive with C2 and C4 (Table 1). These assignments cannot be absolutely confirmed, because we do not have a DNA probe for the region between C4 and V1. Thus, the minimal distance between X3 and X6 is 118 kb (Fig. 5). If X4 and X5 as defined relative to X3 are not the same as the X4 and X5 sites defined relative to X8, there could be additional DNA sequences between the two VC clusters. However, the maximal distance is limited by the 370-kb *Sal*I fragment. Thus, if X8 were just to the left of S2 (Fig. 5), the maximal distance between C4 and V1 could be 196 kb.

Sites X1 and S1 are known on the basis of the cloned DNA. They are susceptible to digestion by *Sal*I and *Xho*I in genomic kidney and J558L DNAs as evident by the 5-kb fragment hybridizing with the 3′V2 probe (Fig. 3). However, with kidney DNA especially, traces of the 60-kb *Xho*I band remain, indicating that the S1 site is methylated and therefore not cut in some of the DNA molecules.

Additional *Xho*I sites were mapped upstream of V2 (Fig. 5). Apparently they, as well as the previously mentioned S4, S3, X1, and S1 sites, are susceptible to digestion in only some of the DNA molecules, making the hybridization patterns with V2 and 3′V2 extremely complex (Fig. 3).

Certain other DNA fragments found with one or several of the probes which represent partial digests with *Xho*I or *Sal*I or both are not discussed in detail in the text. Their interpretation can be obtained from Table 1.

Further analysis of the distance between Vλ1 and C3-C1. Because of the availability of the unique probes 3′V2 and 5′C2 and the presence of the known *Xho*I and *Sal*I sites 3′ of V2 and 5′ of C2, the distance between V2 and C2 was clearly demonstrated by the shortest fragment positive with both of these probes but negative with C2. However, for V1 it was not possible to obtain a unique probe and no sites for rarely cutting restriction enzymes were found on the DNA clones containing the V1 gene. The evidence for placing V1 about 20 kb 5′ of C3-C1 was therefore considered in greater detail. Table 2 summarizes those restriction fragments in kidney and J558L DNAs which overlap the joining sites for V1-J3 and V1-J1 rearrangements. A 57-kb *Xho*I band positive with V2 (=V1) but not C2 defines the distance X5 to X7 on the J558L chromosome carrying the V1-J1 rearrangement. This distance is 80 kb for kidney DNA, indicating a V1-J1 distance of 23 kb (80 − 57).

The distance between V1 and the C2-C1 cluster is also defined by another fragment. With kidney DNA, X4 to X8 was 174 kb (see above). In J558L, the 174-kb fragment was not seen; instead, a 155-kb band positive with V2 (=V1), C2 (=C3), C1, and 3′C1 was apparently derived from the chromosome carrying the V1-J3 rearrangement, making the distance between V1 and J3 19 kb (174 − 155). The additional confirmation of an approximate distance of 20 kb between V1 and J3-J1 with *Sal*I was discussed above (Fig. 2; Table 2; S4 to S2 and S1 to S2).

With the distance between V1 and the C3-C1 cluster apparently so short, it was possible to check the PFGE findings further by direct analysis of genomic DNA digested with enzymes which cut frequently and comparison with the DNA clones. *Xba*I was found to cut V1-containing clones 0.6 kb 5′ of V1 and *Kpn*I and *Sac*I cut within V1. These enzymes did not cut V1-containing clones a second time 3′ of V1 (Fig. 6) (18). Sites for these enzymes were also present in the cloned DNAs representing the most 5′ region upstream of C3-C1 (Fig. 6) (18). When kidney DNA was digested with these three enzymes and Southern blots (32) were probed with V1 (a sequence 3′ of the *Sac*I site within V1), fragments of 7.1 kb (*Xba*I), 10.5 kb (*Sac*I), and 6.5 kb (*Kpn*I) were obtained which all must extend 3′ of V1 because of the origin of the probe (data not shown; Fig. 6). When the cloned DNA maps are aligned with the sizes of these genomic restriction fragments, the genomic 3′ *Kpn*I and *Sac*I sites match such sites in the clones and suggest that only 3.5 kb of DNA has not been cloned between V1 and C3-C1 (Fig. 6). The 3′ *Xba*I

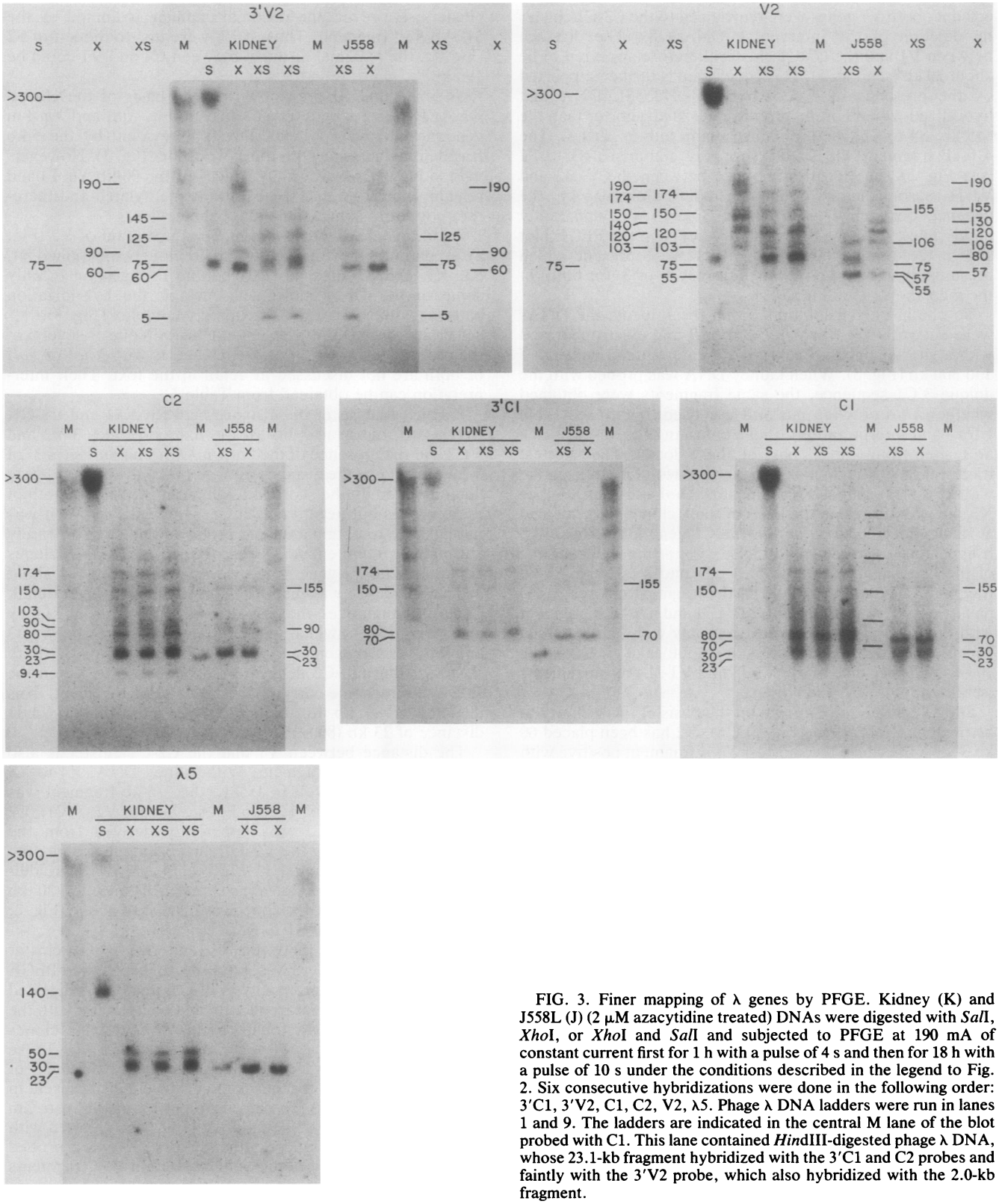

FIG. 3. Finer mapping of λ genes by PFGE. Kidney (K) and J558L (J) (2 μM azacytidine treated) DNAs were digested with *Sal*I, *Xho*I, or *Xho*I and *Sal*I and subjected to PFGE at 190 mA of constant current first for 1 h with a pulse of 4 s and then for 18 h with a pulse of 10 s under the conditions described in the legend to Fig. 2. Six consecutive hybridizations were done in the following order: 3′C1, 3′V2, C1, C2, V2, λ5. Phage λ DNA ladders were run in lanes 1 and 9. The ladders are indicated in the central M lane of the blot probed with C1. This lane contained *Hin*dIII-digested phage λ DNA, whose 23.1-kb fragment hybridized with the 3′C1 and C2 probes and faintly with the 3′V2 probe, which also hybridized with the 2.0-kb fragment.

site found upon digestion of kidney DNA would be located just 5′ of the C3-C1 clones. The presence of the *Kpn*I and *Sac*I sites in the C3-C1 clones 4 kb apart, as predicted from the genomic Southern blot, and the calculated gap between the clones sufficiently large to include the 3′ *Xba*I site found with cell DNA support the conclusions drawn from the PFGE analysis. Thus, the V1-J3 distance appears to be about 19 kb (3 kb 3′ of the V1 present in genomic clones [18]

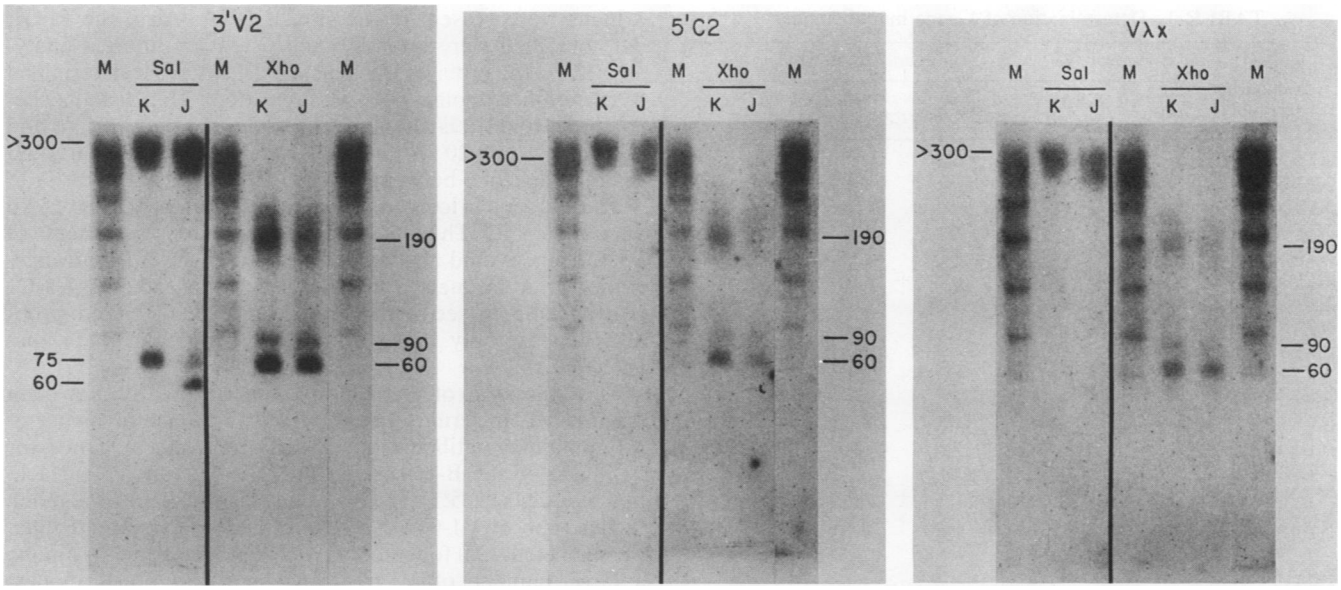

FIG. 4. Mapping of Vλx. Kidney (K) and J558L (J) (2 μM azacytidine treated) DNAs were digested with *Sal*I or *Xho*I and subjected to PFGE at 4 s for 1 h and 10 s for 20 h under the described conditions in the legend to Fig. 2. Hybridization was consecutive with VX, 3'V2, and 5'C2. The M lanes contained phage λ DNA ladders. The numbers to the sides indicate molecular sizes in kilobases.

+ a 3.5-kb gap + 12 kb 5′ of the J3 present in genomic clones [18] = 18.5 kb).

Mapping the location of Vλx. A new Vλ gene, Vλx, has been recently discovered (6, 28). To map its location relative to the other λ genes, PFGE blots of DNA cut with *Sal*I or *Xho*I were hybridized with a Vx probe (Fig. 4). The *Xho*I pattern was found to be identical to the hybridization patterns obtained with the 3′V2 and 5′C2 probes, indicating that Vx is located between X1 and X2 (Fig. 5). The *Sal*I pattern lacks the 75- and 60-kb bands seen with 3′V2. Therefore, Vx must lie 3′ of S1. To obtain a finer map, genomic clones of kidney DNA (18) were digested with a variety of restriction enzymes, blotted, and probed with Vx (data not shown). In this way, it was determined that Vx is located 19 kb 3′ of V2. It is present on a 1-kb *Bam*HI fragment located within a 10.5-kb *Eco*RI fragment. Its transcriptional orientation must be the same as that of V2 for the following reasons. Rearrangement of Vx to J2 results in a 7.6-kb *Hin*dIII fragment (29), there is a *Hin*dIII site 0.1 kb 3′ of J2, and there are *Hin*dIII sites 7.2 kb 5′ and 2.5 kb 3′ of Vx relative to the V2 transcriptional orientation. The 7.2-kb *Hin*dIII site must be upstream of Vx to give 7.2 kb 5′ of Vx + 0.3 kb for the Vx gene + 0.1 kb 3′ of J2 = 7.6 kb. Thus, V2 and Vx must be in the same transcriptional orientation, and Vx and J2 rearrange by deletion of the intervening DNA, like the other λ genes.

λ5 is outside the V2-to-C1 region. A recently discovered immunoglobulinlike gene, λ5, has some sequence homologies with Jλ and Cλ genes (15, 27). Although it is expressed in pre-B cells, it is apparently not rearranging (15). Its presence on chromosome 16 (14) made it interesting to determine whether it is located between the true λ genes. The PFGE blot tested with other λ probes was therefore rehybridized with λ5 (Fig. 3). With *Sal*I alone, a 140-kb fragment was obtained for kidney DNA rather than the 75- or 370-kb fragments seen with the other λ probes. Therefore, λ5 cannot be located between S4 and S2. Furthermore, λ5 is apparently not within the left portion of the map in Fig. 5, because the 125-kb fragment seen with *Sal*I and *Xho*I and the V2 or 3′V2 probe (X11 to S1) does not hybridize with λ5. Thus, λ5 must lie outside the approximately 500-kb DNA sequence in and around the other known λ genes.

DISCUSSION

The results of these PFGE maps show that in the original cloning (18) of V1 and C3-C1 genes the two subloci were almost linked. For the cloning of λ DNA, phage or cosmid clones were selected by hybridization with V1 or C1 (18). In the C3-C1 locus, clones extending about 12 kb 5′ of C3 were obtained (18). However, for V1 no clones were obtained which extended beyond 3 kb 3′ of V1 (18). Furthermore, far fewer of the clones recovered contained V1 than any other λ gene. It was presumed that some "poison" sequence inhibiting bacteriophage or *Escherichia coli* growth was located beyond 3 kb 3′ of V1. Perhaps this sequence contains a site

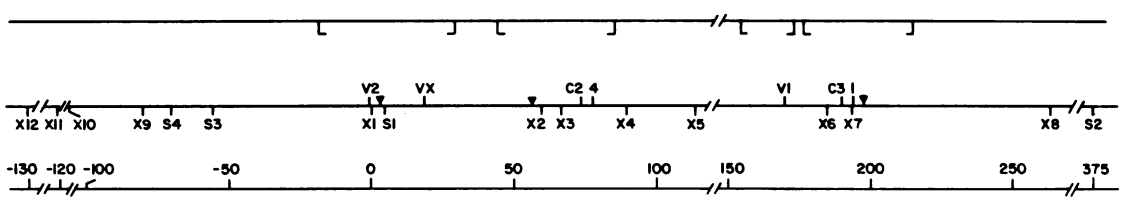

FIG. 5. Linkage map of mouse λ genes. Refer to Table 1 for interpretation of the PFGE blot hybridizations. X, *Xho*I; S, *Sal*I. The inverted triangles indicate (from left to right) the 3′V2, 5′C2, and 3′C1 probes. The brackets at the top show the limits of the cloned genomic DNA (17, 18, 31). The numbers at the bottom indicate molecular sizes in kilobases.

TABLE 1. Determination of λ gene map distances[a]

Fragment endpoints (size [kb])	Cleavage by:			Hybridization with:						
	*Sal*I	*Xho*I	*Xho*I-*Sal*I	V2	3′V2	Vx or 5′C2	C2	C4[b]	C1	3′C1
X13-X1 (140)		+		+						
X13-S1 (145)			+	+	+					
X12-X1 (130)		+		+						
X11-X1 (120)		+		+						
X11-S1 (125)			+	+	+					
X11-X3 (190)		+		+	+	+				
X10[c]-X1 (106)		+	+[d]	+						
X9[c]-X1 (80)		+		+						
S4-S1 (75)	+		+[d]	+	+					
S4-S2 (445)	+			(+)	+	+	(+)		(+)	+
S3-X1 (55)			+	+						
S3-S1 (60)	+			+	+					
X1-S1 (5)			+		+					
X1-X2 (60)		+	+[d]	+	+					
X1-X4 (90)		+	+[d]		+	+	+	+	[+]	
S1-S2 (370)	+			[+]		+	(+)		(+)	+
X2-X4 (30)		+	+				+	+	[+]	
X3-X4 (23)		+	+				+	+	[+]	
X3-X5 (47)[e]		+	+				+	+		
X3-X8 (~200)		+	+	[+]			(+)	+	(+)	+
X4-X7 (103)		+	+	[+]			[+]			
X4-X8 (174)		+	+	[+]			[+]		+	+
X5-X8 (150)		+	+	[+]			[+]		+	+
X6-X7 (9.4)		+	+				[+]			
X6-X8 (80)		+	+				[+]		+	+
X7-X8 (70)		+	+						+	+

[a] This table is a compilation of the data shown in Fig. 2 to 4. Tabulation is from left to right according to the map in Fig. 5. Brackets denote a V2 probe that hybridized to a V1 gene, C2 to C3, or C1 to C4. Parentheses indicate probes that hybridized to both cross-hybridizing genes.

[b] Not shown in a figure.

[c] These sites were digested only in J558L.

[d] Bands were seen with *Xho*I and *Sal*I in addition to *Xho*I alone, because certain *Sal*I sites were not cut or were incompletely cut.

[e] This band was seen only with J558L in a blot not shown.

for a unique restriction enzyme present in the packaging extracts or bacterial hosts used (24, 26). Thus, clones containing this region would have been digested and rendered unpackageable. It would be possible to test this idea by producing phage clones packaged in extracts and grown in *E. coli* strains which are free of such restriction enzymes and searching for the existence of V1-C3-C1 clones.

The λ map derived by PFGE analysis is likely to be correct, because it conforms in large stretches to the maps of clones of germ line DNA (Fig. 5, brackets); thus, except for X4 and X5, all *Xho*I and *Sal*I sites found by PFGE within the λ locus were present in the cloned DNA. Also, the PFGE data obtained were internally consistent in different analyses. Thus, the order of the genes, V2-C2-V1-C1, determined by deletion mapping (18), is supported. The distance between V2 and C2 is about 74 kb, while that between V1 and C3 is only about 20 kb. C4 and V1 are about 92 kb apart, and thus the distance between V2 and C3 is about 190 kb.

TABLE 2. DNA restriction fragments which are shorter in J558 DNA than in kidney DNA

Fragment endpoints	Hybridizing probes[a]	Fragment size (kb)		Difference (kb)	Presumed rearrangement in J558L
		Kidney	J558L		
S4-S2	All	445	425	20	V1-J3, V1-J1
S1-S2	All except 3′V2	370	350	20	V1-J3, V1-J1
X4-X8	[C2], [V2], C1, 3′C1	174	155	19	V1-J3
X5-X7	[V2]	80[b]	57	23	V1-J1

[a] Brackets indicate that the C2 probe hybridizes with C3 and the V2 probe hybridizes with V1.

[b] For kidney DNA, the X5-X7 distance was calculated as 150 kb (X5-X8) − 70 kb (X7-X8).

The Vx gene is located 19 kb downstream of V2 and 55 kb upstream of C2. Thus, besides the known rearrangements of Vx-C2, one would also expect to find Vx–C3-C1 rearrangements at a frequency comparable to that of V2–C3-C1 rearrangements (see below). These were not observed, probably because only 10 Vx-producing hybridomas were analyzed (6, 28).

The expression of Vλ1 and Vλ2 genes is greatly skewed in favor of V1. In serum, the ratio is 9:1 (7). This prevalence of V1-containing antibodies may partly represent selection for λ1, since on the B-cell level the V1-to-V2 ratio of surface-positive cells is 5:1 (7). However, in pre-B cells a much higher ratio of V1-to-V2 rearrangements is apparent; thus, Persiani et al. (22) found only two V2 rearrangements among a large number of V1 rearrangements in pre-B clones. Presumably, pre-B cell clones reflect the actual rearrangement frequencies without selection of the product. The rearrangement of Vx to C2 is slightly more frequent than that of V2 to C2 (28). Comparing these rates of rearrangement with the distances between the V and C genes shown in Fig. 5, one finds an inverse correlation; V1–C3-C1 rearrangements, which connect a distance of only about 20 kb, are much more frequent than V2-C2 or Vx-C2 rearrangements, which cover stretches of 55 or 75 kb; V2–C3-C1 rearrangements across about 190 kb are extremely rare. Apparently, the recombination process favors proximate partners. This conclusion is compatible with the finding of preferential VD joining of the most D-proximal VH genes (21, 37). The preference for proximate joining partners may support a tracking model of immunoglobulin gene rearrangement (37), although in certain procaryotic recombination systems a tracking model is unlikely because a continuous DNA stretch between the targets is not required (5). Distances of 0.9 to 30 kb do not seem to influence rearrangement rates of T-cell receptor genes (4). Thus, if the immunoglobulin–T-cell receptor gene recombinase (36) operates in a tracking mode, its progression rate must be too high to permit a negative effect of short and intermediate distances. Alternatively, the effect of the long distances, as in the Vλ2-Cλ2 and Vλ2–Cλ3-Cλ1 rearrangements, may relate to the lowered chance for very distant DNA sequences to meet. It also remains to be determined how the actual sequences involved modulate the distance effect. Except for the predominantly rearranging elements of the chicken λ genes, which are very closely spaced (25), no immunoglobulin gene locus has been mapped in its entirety. It is known that VH genes are spaced at a distance of about 10 to 30 kb and that at least 100 VH genes exist (11). Thus, there are at least 1,000 kb between D and the last VH gene. Nevertheless, the distant VH genes are represented among B cells. Perhaps there is a stronger selection pressure on expressed VH genes than on the expression of λV2-J2, Vx-J2, and V2–J3-J1 rearrangements. Alternatively, λ gene rearrangement may, for some structural reason, be a marginally efficient process. At least in mice, the λ/κ ratio is very low (7). The rearrangement recognition sequences of λ deviate from consensus sequences (19). Such structural constraints may then be exaggerated if the joining gene segments are a greater distance apart.

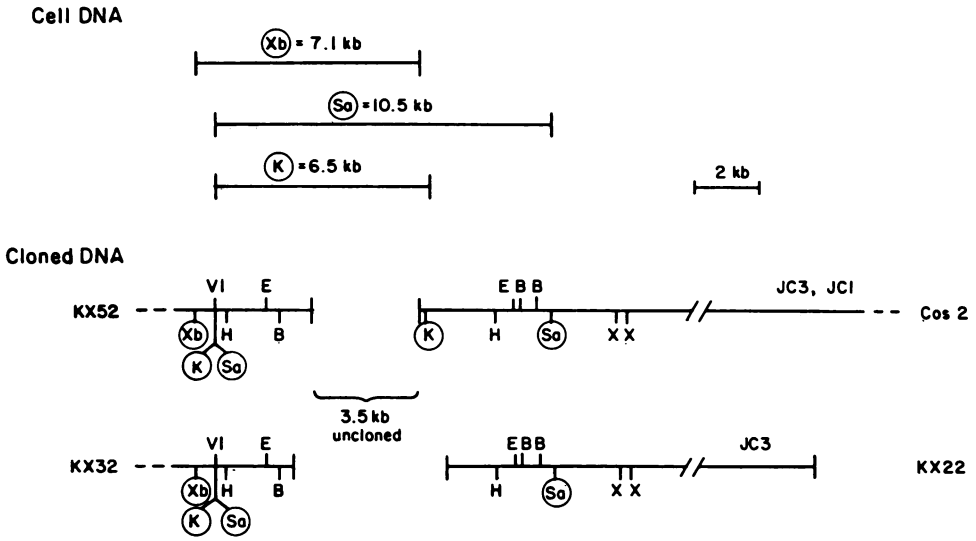

FIG. 6. Comparison of restriction sites between Vλ1 and JCλ3 in cloned and cell DNAs. KX52 and KX32 are two phage clones containing Vλ1 (V1); Cos 2 and KX22 are JC3-containing cosmid and phage clones which extend most 5′ (18). Xb, *Xba*I; Sa, *Sac*I; K, *Kpn*I; H, *Hin*dIII; E, *Eco*RI; B, *Bam*HI.

Lambda genes are unusual in several respects, such as the control of their expression (8). In contrast to other immunoglobulin genes, no transcriptional enhancer has been found for λ (23). Knowing the linkage map of λ genes will, we hope, aid in the analysis of the function of this interesting locus.

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