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Genetics

Deletions of κ chain constant region genes in mouse λ chain-producing B cells involve intrachromosomal DNA recombinations similar to *V–J* joining

(B-cell ontogeny/*RS* DNA chromosomal assignment/DNA sequence)

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ABSTRACT **We isolated and characterized the germ-line counterpart of a DNA segment designated *RS* (for recombining sequence), that is frequently recombined in mouse λ light chain-producing B lymphocytes. Using Southern blot analyses of myelomas and mouse–Chinese hamster fusion cell lines, we found that *RS* DNA sequences are located on mouse chromosome 6, evidently more than 15 kilobases downstream of the κ light-chain locus. We find that a typical recognition site for Ig gene recombination is situated within germ-line *RS* sequences near the recombination points observed in at least two λ chain-producing cell lines. This represents a complete and functional Ig recognition site that is not directly associated with Ig genes. We also characterized a recombined RS segment isolated from the cell line BM18-4.13.9. This recombined segment has a variable region κ light chain gene (V_κ) joined directly to *RS* sequences. Our results suggest that the deletion of the κ light chain constant region (C_κ) exon in many λ chain-producing B cells is the result of *RS* recombination and that C_κ deletion may be mediated by the same processes as antibody gene *V–J* joining (*J* = joining segment gene). We discuss the potential biological significance of *RS* DNA recombination in B-cell maturation.**

Immunoglobulin proteins are comprised of two identical heavy and two identical light chains. In the germ-line genome, the variable (V) and constant (C) portions of each of these chains are encoded by gene segments that are widely separated. During the maturation of antibody-producing B cells, these gene segments are brought together by DNA recombination events to form a functional gene (1). Among mouse serum antibodies, two classes of light chains, κ and λ, are found. Within an individual antibody, however, only one class, either κ or λ, is present, and only one class appears to be expressed in an individual B lymphocyte. During B-cell differentiation, the recombination of light chain genes appears to be developmentally regulated. Two lines of evidence suggest that recombination of κ chain genes occurs before recombination of λ chain genes. First, in κ chain-producing cell lines, λ genes are usually not recombined, whereas in λ chain-producing cell lines, κ genes are frequently recombined or deleted (2–4). Second, in Abelson virus-transformed cell lines that undergo κ gene recombination in tissue culture, no λ gene recombinations are observed (5). These observations have led to the hypothesis that, in a maturing B cell, λ chain gene recombinations are only activated when κ chain gene recombinations fail to produce a functional light chain gene (6). The regulatory signals that might mediate this "switch" from κ to λ chain gene recombination have not yet been elucidated.

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We have reported that two λ chain-producing cell lines, MOPC315 and CH2, display novel DNA recombinations within the κ chain locus different from all previously observed κ chain gene recombination events (7). In these two cell lines, a segment of DNA [designated *RS* for recombining sequence] has recombined into the J_κ–C_κ intron region and replaced the C_κ exon (J_κ = κ chain joining region gene; C_κ = κ constant region gene). In addition to being recombined in MOPC315 and CH2, *RS* DNA also was found to be recombined frequently in λ chain-producing, but not in κ chain-producing, hybridomas. Although no functional role for *RS* DNA has yet been demonstrated, these results raised the possibility that *RS* recombinations might be involved in the switch from κ to λ chain gene recombination in maturing B cells.

In this article we report that germ line *RS* sequences are located on mouse chromosome 6 and that, within germ-line *RS* DNA, an Ig gene recognition site is located contiguous to the sites of *RS* recombination observed in MOPC315 and CH2. We also have detected and characterized a recombined *RS* segment that contains a V_κ gene joined directly to *RS*.

MATERIALS AND METHODS

DNA Clones. A mouse germ-line DNA library (8) was donated by J. Miller and U. Storb (University of Washington). A probe specific for *RS* sequences (rs0.8) was prepared from a recombined *RS* clone (λ1059.M315-rs1) (7). The rs0.8 probe was used to isolate a germ-line *RS* clone (Ch4A.Kd-rs1). A recombined *RS* clone (λ1059.BM-rs1) was also isolated from a λ1059 (9) library of the cell line BM18-4.13.9. For maps of the various clones and locations of the probes utilized, see Fig. 2.

Somatic Cell Hybrids. DNAs from mouse–Chinese hamster hybrid cell lines were provided by D. Pravtcheva and F. Ruddle (Yale University). The mouse parental cells for the hybrids were BALB/c sarcomas MethA (for MAE32, 4, 6A, 8C, and 9A), CMS4 (for TUCE 12G/9), and CI4(s) (for CEC); BALB/c embryo fibroblasts (for MFE2/3, 2/1/7, 2/1/2, 10 and 8); and A/HeJ macrophages (for BEM1-6, EcM4e, 3B3, 4AG4, and 4B3/AZ3).

Southern Blots and DNA Sequencing. Mouse *RS* sequences were detected by filter hybridization methods (10–12) using cell hybrid DNAs isolated at the same cell passage as that used for karyotype and marker enzyme analyses (13). Filters were washed at 65°C in 0.015 M NaCl/0.0015 M sodium citrate, pH 7. DNA sequences were determined by the dideoxynucleotide chain-termination method (14) with phage M13 or pUC sequencing vectors (15, 16).

Abbreviations: V, C, and J, variable, constant, and joining regions; V_κ, J_κ, and C_κ, genes for κ light chain V, J, and C regions; C_β and V_β, genes for T-cell receptor β chain C and V regions; kb, kilobase(s); bp, base pair(s).

RESULTS

***RS* Sequences Are Located on Mouse Chromosome 6.** To determine the chromosomal location of the *RS* sequences in mouse, we analyzed interspecies somatic cell hybrids by Southern filter hybridization with a mouse *RS* DNA probe. The Chinese hamster cell line E36 was used as one parent for all of the 17 somatic cell hybrids that were analyzed. The 6 hybrid lines scoring positive for *RS* localize *RS* sequences to mouse chromosome 6 (Table 1). The strongest case against localization to any other chromosome is the CEC cell line DNA (Fig. 1), which contains only a single translocated mouse chromosome t(X;6). We determined that the portion of chromosome 6 retained in CEC DNA contains J_κ and C_κ regions (data not shown). Caccia *et al.* (17) also have shown that CEC DNA contains the J_κ region and the gene for the β chain of the T-cell receptor. Furthermore, in MFE 10, only chromosome segments centroproximal to band C2/C3 are present, whereas in CEC only chromosome 6 segments distal to bands B1/B2 are present (13). These data indicate that the J_κ–C_κ region, *RS* DNA, and the T-cell receptor β-chain gene region all reside between bands B1/B2 and C2/C3 on mouse chromosome 6.

An Immunoglobulin Gene Recognition Site Is Found Within Germ-Line *RS* Sequences. We isolated a 17-kilobase (kb) clone containing germ-line *RS* sequences (Kd-rs1) from BALB/c kidney DNA. Comparisons of the Kd-rs1 clone to the recombined *RS* DNA clones from MOPC315 and CH2 indicated that the germ-line *RS* clone spans the *RS* recombination points observed in MOPC315 (Fig. 2) and CH2 (not shown).

We previously have shown that *RS* recombinations in MOPC315 and CH2 occur upstream of an Ig recognition heptamer within the J_κ–C_κ intron (7). We now have sequenced 1390 base pairs (bp) of germ-line *RS* surrounding the recombination points in MOPC315 and CH2. The sequence data show an Ig recognition site within germ-line *RS* DNA (Fig. 3) that is contiguous to the *RS* recombination points

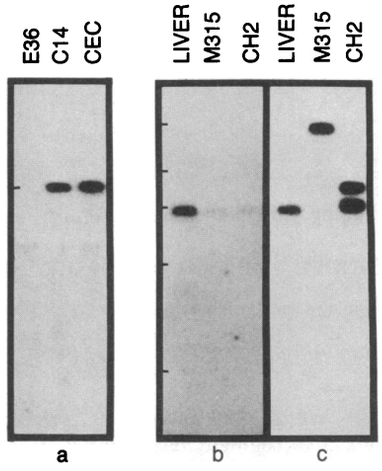

FIG. 1. Southern blots of genomic DNAs probed with *RS* DNA. (*a*) *RS* sequences in a mouse–hamster hybrid cell line. A Southern blot of ≈25 μg of *Eco*RI-digested genomic DNAs that were hybridized with the rs0.8 probe (see Fig. 2). CEC is a hybrid cell line resulting from the fusion of the Chinese hamster line E36 and the mouse sarcoma CI4. It contains a portion of chromosome 6 translocated to the X chromosome and no other mouse chromosomes. The bands detected with the *RS* DNA probe migrate at 6.5 kb as indicated. (*b* and *c*) RS recombinations in B-cell lines. *Eco*RI-digested DNAs (20 μg each) from the indicated cell lines were probed with the rs0.4 fragment (*b*) and later rehybridized with the rs0.8 clone (*c*). Positions of *Hin*dIII-cut phage λ DNA marker bands (23, 9.4, 6.6, 4.4, 2.3, and 2.0 kb, respectively) are indicated.

Table 1. Chromosomal localization of *RS*

Cell line	Chromosome complement																				Scoring		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y	J_κ	C_κ	*RS*
BEM 1-6	+	+	+	+	−	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	−	ND	ND	+
MFE 2/3	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+*	ND	+
MFE 2/1/7	+	+	+	−	−	+	+	+	+	−	−	+	+	−	+	−	+	−	+	+	−	ND	+	+
MFE 2/1/1	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	+*	+	+
MFE 10	+	+	R	−	−	R	+	+	+	−	#	R	+	+	+	+	+	+	−	+	−	ND	+	+
CEC	−	−	−	−	−	R	−	−	−	−	−	−	−	−	−	−	−	−	−	R	−	+	ND	+
3B3	−	+	−	−	+	−	+	−	+	−	−	+	+	+	+	−	+	+	+	+	−	ND	ND	−
4AG4	+	+	−	−	−	−	+	−	−	−	−	+	−	−	+	−	+	−	+	+	−	ND	ND	−
EcM4e	−	−	−	−	−	−	−	−	−	−	−	−	−	R	+	−	−	−	−	−	−	ND	ND	−
MAE 32	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	ND	ND	−
MAE 4	−	−	−	−	−	−	−	−	−	−	−	R	−	−	−	−	−	−	−	+	−	ND	ND	−
MFE 8	R	+	+	R	+	−	+	R	+	+	#	+	+	+	+	+	+	+	+	+	−	ND	ND	−
TUCE 12G/9	−	+	−	−	+	R	−	R	−	+	−	+	+	+	+	+	+	−	+	+	−	ND	−	−
4B3/AZ3	−	+	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−	+	−	−	ND	ND	−
MAE 6A	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	ND	ND	−
MAE 8C	−	−	−	−	−	−	−	−	−	−	−	R	−	−	−	−	−	−	−	R	−	ND	ND	−
MAE 9A	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	ND	ND	−
Discordancy fraction	4/16	6/17	2/16	3/16	9/17	0/16	6/17	1/15	4/17	5/17	4/15	7/14	4/17	6/16	7/17	8/17	5/17	4/17	7/17	8/15	6/17			

To ascertain the chromosomal location of *RS* DNA, a series of cell lines generated by fusing Chinese hamster cells with mouse cells were hybridized with the rs0.8 probe as described in Fig. 1. The karyotype of each of the 17 hybrids tested is given. The column at the right indicates positive or negative scoring for the presence or absence of *RS*, J_κ, and C_κ. The discordancy fraction under each chromosome refers to the number of hybridization experiments that disagree with assignment of *RS* to that chromosome divided by the number of trials for that chromosome. Rearranged chromosomes and chromosomes present in <15% of the cells tested were not included in the calculation of the discordancy fraction except for the portions of chromosome 6 present in the MFE 10 and CEC lines. The MFE 10 and CEC lines were included in the calculations because, by Southern hybridization, these lines were positive for another gene mapping to chromosome 6 (either J_κ or C_κ). The rearranged chromosome 6 detected in TUCE 12G/9 scored negative for C_κ. +, Present; −, not present; ND, not determined; R, recombined chromosome; #, present in <15% of the cells tested.

*Caccia *et al.* (17).

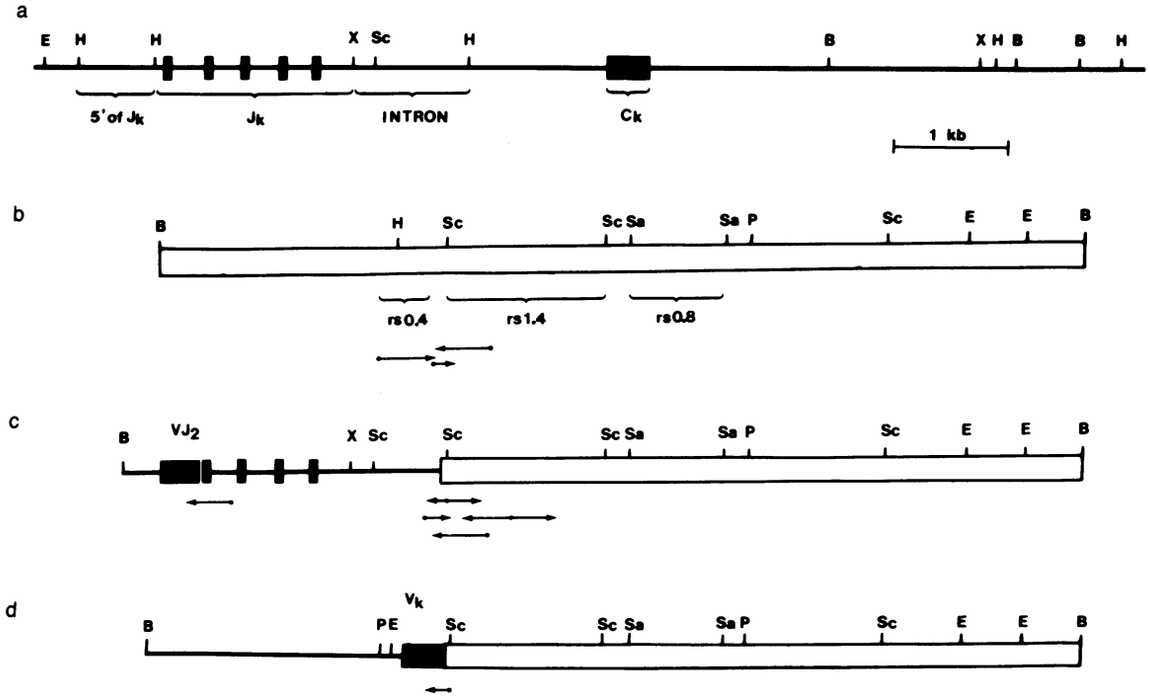

FIG. 2. Restriction enzyme maps of *RS* DNA clones. (*a*) Restriction sites within the germ-line J_κ–C_κ locus. (*b*) Restriction sites within an 8-kb *Bam*HI germ-line *RS* segment and hybridization probes that were used in these studies. (*c*) The restriction map for the recombined *RS* DNA in MOPC315. (*d*) The restriction map for the recombined *RS* DNA in BM18-4.13.9. V_κ gene segments and segments of DNA derived from the J_κ–C_κ locus are indicated as solid bars, whereas DNA segments derived from *RS* are indicated as open bars. The arrows in *b*, *c*, and *d* indicate regions that have been cloned into M13 mp9 or pUC13 and sequenced. All maps are drawn to scale. Restriction enzymes were: *Bam*HI (B); *Eco*RI (E); *Pvu* II (P); *Hin*dIII (H); *Sau*3A (Sa); *Sac* I (Sc); and *Xba* I (X). Not all *Sau*3A sites have been mapped in *RS* DNA.

found in MOPC315 and CH2. The *RS* recognition site has a heptamer that is (*i*) identical to the consensus heptamer of J_κ and (*ii*) correctly spaced 23 bases from a nonamer sequence that contains six of the nine conserved bases found among Ig recognition nonamers. It is striking that the recombination between the intron of the J_κ–C_κ locus and *RS* DNA resembles V_κ–J_κ recombination in the use of the appropriate recognition sequences. Thus, it would appear that Ig recognition sequences direct *RS* recombination. Fig. 4 depicts normal V_κ–J_κ joining and *RS* recombination, showing the similarity of these two types of events.

We searched the *RS* sequence to locate other possible Ig or T-cell receptor recognition sequences. We found one additional V_κ recognition heptamer at position 518, one J_β (*J* region of T-cell receptor β chain gene) recognition heptamer at position −87, and a V_β (*V* region of T-cell receptor β chain gene) heptamer at position 823. However, no nonamers were found with these haptamers, indicating that only one complete recognition site is present within the *RS* sequence.

V_κ Gene Segments Can Recombine Directly to *RS* DNA. For normal Ig genes, *V–J* and *V–D–J* (D = diversity region gene) joinings take place only between gene segments having recombination signals with differently sized spacer segments [the 11–22 rule (18–21)]. Recombination appears to require one recognition site with a 11- or 12-nucleotide spacer between the conserved heptamer and nonamer and another site with a 22- to 24-nucleotide spacer. As discussed above, our *RS* sequence data show a 23-nucleotide spacer recognition site. Thus, in accordance with the 11–22 rule, we might expect that direct V_κ–*RS* joinings could occur. Direct V_κ–*RS* joining could result in the loss of the entire J_κ–C_κ region, explaining the absence of that region that is observed in many cell lines (3, 4, 7). To determine the nature of an *RS* recombination that was not associated with the J_κ region, we isolated a recombined *RS* segment (λ1059.BM-rs1) from a subclone of the Abelson virus-transformed pre-B-cell line

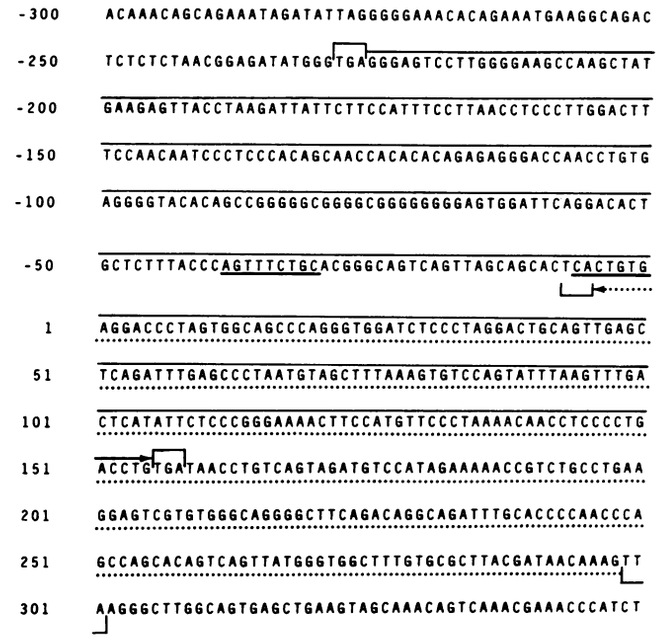

FIG. 3. Sequence analysis of germ-line *RS* DNA. Nucleotide sequences extending 300 bp upstream and 300 bp downstream of the MOPC315 recombination site are shown. The first base downstream of the Ig recognition heptamer is chosen as the +1 reference point. The heavy black underlines indicate an Ig recognition heptamer and nonamer. The solid arrow above the sequence indicates a protein open reading frame, with the arrow pointing in the 5′–3′ direction of DNA strand. The dotted arrow below the sequence indicates another protein open reading frame of 99 amino acids that is found on the opposite strand. The open reading frames are defined only by stop codons (brackets).

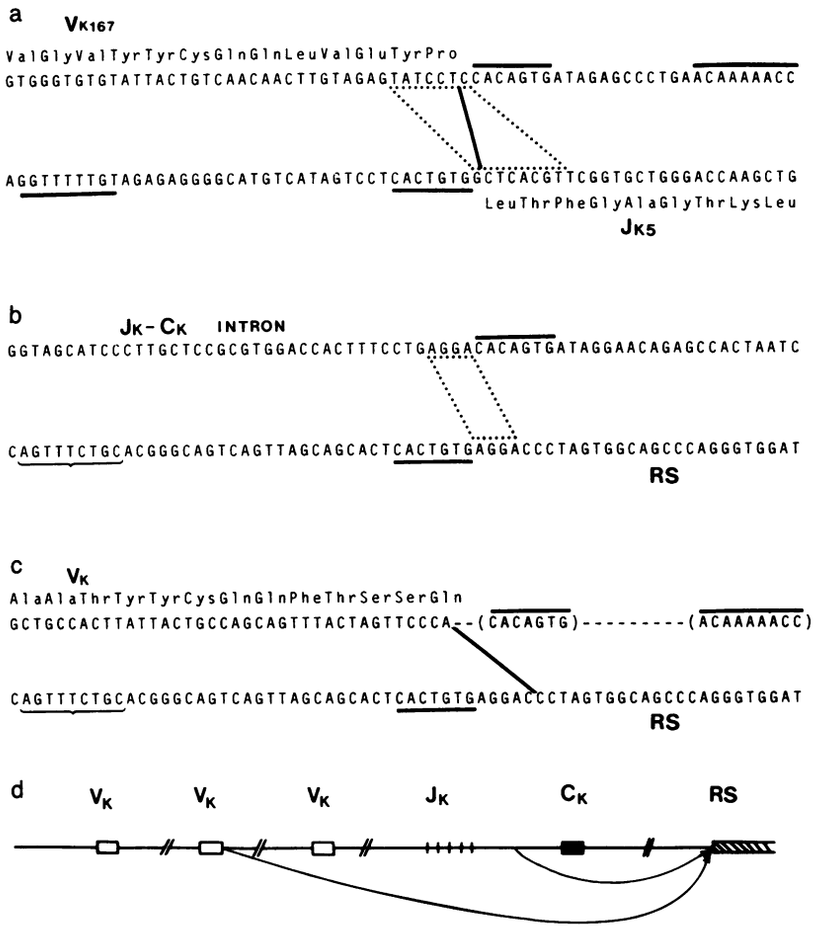

FIG. 4. Comparison of *V–J* joining and *RS* recombination. (*a*) An example of *V–J* joining between $V_{\kappa 167}$ and $J_{\kappa 5}$. The dotted lines indicate the region in which *V–J* joinings have been found, while the solid vertical line shows the recombination site in MOPC167. (*b*) A model for the recombination of *RS* DNA with the J_κ–C_κ intron. The dotted lines delineate the possible sites of recombination. The exact site cannot be determined because the sequence A-G-G-A is found at the recombination site in both *RS* DNA and the J_κ–C_κ intron (18). (*c*) Recombination between V_κ and *RS*. The dashes represent undetermined germ-line V_κ sequences and the presumed V_κ recognition sequences are in parentheses. The solid diagonal line shows the *RS* recombination site in BM18-4.13.9. In *a*, *b*, and *c*, the heavy underlines indicate the Ig recognition heptamers and nonamers. The brackets in *b* and *c* indicate possible nonamers. (*d*) The organization of V_κ (open bars), J_κ–C_κ (solid bars), and *RS* (hatched bars) on chromosome 6 is shown. Arrows indicate the types of *RS* recombination characterized.

BM18-4 (22). Southern blot analysis indicated that this cell line (BM18-14.13.9) has lost the J_κ–C_κ region from its genome (data not shown). The DNA sequences adjacent to the *RS* recombination point in BM-rs1 were determined (Fig. 2*d*). The *RS* recombination point in this clone is located within five bases of the *RS* recombination sites that are observed in MOPC315 and CH2 (Fig. 4*c*). However, the DNA segment that recombined with the *RS* DNA in BM-rs1 is not the J_κ–C_κ intron. To characterize the recombined DNA segment from BM-rs1, we sequenced 150 nucleotides adjacent to the *RS* recombination point and decoded this sequence to amino acids. In one reading frame, these sequences encode a continuous stretch of 50 amino acids. Comparing this reading frame to the most common amino acids found in κ light chains (23), we determined that all 13 of the invariant residues and many of the conserved residues that occur within the 3′ portion of V_κ regions are encoded within this 50 amino acid open reading frame (data not shown). We conclude that, in BM18-4-13.9, a V_κ segment has recombined directly to *RS* (Fig. 2*d*). Because 8 of the 10 *RS* recombinations that we had previously found in B-cell tumors did not hybridize with J_κ probes (7), most *RS* recombinations do not involve the J_κ–C_κ region and may, in fact, represent direct V_κ–*RS* joining.

***RS* DNA Is More Than 15 kb Downstream of the C_κ Gene.** The mapping of *RS* to mouse chromosome 6, the deletion of C_κ in MOPC315 and CH2, the deletion of J_κ–C_κ in BM18-4.13.9, and the structures of recombined V_κ–J_κ–*RS* and V_κ-*RS* segments in these cell lines, when considered together, suggest that *RS* may lie downstream of the C_κ exon in the germ line. However, we found no detectable homologies between the germ-line Kd-rs1 clone and a germ-line clone of the J_κ–C_κ region (Charon 4A.Sp101; ref. 24). As the Sp101 clone extends 10 kb downstream of the C_κ exon and the Kd-rs1 clone extends ≥5 kb on either side of the *RS* recombination site, it appears that *RS* is located >15 kb downstream of the C_κ exon. We also sought to determine the organization of the κ-chain locus and *RS* relative to the T-cell receptor gene region. We probed Southern blots of DNAs from B-cell lines that had undergone *RS* DNA recombination with probes specific for the *C* region of the T-cell receptor β chain gene. In all cases examined, the T-cell receptor genes were neither deleted nor recombined. We also examined Southern blots of DNAs from T-cell lymphomas, which have recombined T-cell receptor genes, for the presence of *RS* DNA and discovered neither deletion nor recombination of *RS* DNA (data not shown). We conclude that the murine T-cell receptor β-chain gene region is either 5′ of V_κ or 3′ of *RS*.

Chromosomal Deletions Accompany *RS* Recombination. To determine what happens to sequences upstream of the *RS* recombination point after the *RS* recombination event, a fragment of *RS* DNA (rs0.4) that extends from position −511 to −93 (Fig. 2*b*) was isolated from Kd-rs1 to probe the Southern blot shown in Fig. 1*b*. DNAs from the cell lines MOPC315 and CH2, which have a recombined *RS* band as shown by the rs0.8 probe (Fig. 1*c*), failed to show any bands with the upstream rs0.4 probe, thus indicating that the upstream *RS* DNA is deleted in these cell lines. These results augment the finding that C_κ is also deleted in MOPC315 and CH2 (7) and support the contention that *RS* is downstream of the κ chain locus. In addition, complete lack of both C_κ and rs0.4 sequences in CH2 and MOPC315 is consistent with a deletion mechanism for *RS* DNA recombination. However, our results cannot exclude the possibility that *RS* recombination events could be the result of sister chromatid exchange because it is not possible for us to follow individual daughter cells after the *RS* recombination event. It could also be

possible that the mechanism of *RS* recombination involves an interchromosomal exchange. However, because interchromosomal exchange does not occur during *V–J* joining and because *RS* recombination resembles *V–J* joining, we do not consider this last mechanism likely.

RS DNA Does Not Encode Any Previously Known Genes. We searched the sequence of our *RS* DNA for all potential protein open reading frames and found two large open reading frames, one of which was a potential 101-amino acid segment that approximates the correct size for a V region and extends from DNA position 298 to the *RS* recombination point (dotted line in Fig. 3). It was compared to protein sequences in the Gene Bank Data Base, and no significant homology to any known protein was found. Recent work has shown that there is a great deal of heterogeneity among T-cell receptor V_β genes (25). Considering that the κ chain locus, the T-cell receptor β chain gene region, and *RS* DNA all reside on chromosome 6, the possibility that *RS* recombinations represent aberrantly recombined V_β regions was explored more extensively. Direct comparison with all currently published V_β regions failed to show any regions of significant homology. Based on these lack of protein sequence homologies, we conclude that this *RS* reading frame is neither a V_κ gene nor any T-cell receptor V_β region.

Our sequence analysis also showed a second open reading frame within the germ-line *RS* sequences that extends from −226 to +156 and potentially codes for 127 amino acids (solid line in Fig. 3). This open reading frame is disrupted at amino acid 75 by the *RS* recombinations in MOPC315, CH2, and BM18-4.13.9. In addition, there are several short open reading frames of 40–70 amino acids throughout this region of *RS* DNA that were found by translating either one or the other DNA strand. We did not find clear RNA processing signals or polyadenylylation sites associated with any of these open reading frames; thus, their role as potential proteins or exons was not apparent.

DISCUSSION

We have reported previously that *RS* recombination occurs frequently in λ chain-producing B-cell lines. We demonstrated in this paper that *RS* DNA resides on chromosome 6, on which the κ chain locus is also found, and that *RS* recombinations are associated with intrachromosomal and not interchromosomal rearrangements. Our results suggest that *RS* recombination events closely resemble *V–J* joining and may be utilizing κ chain antibody gene recombinases. The organization of *RS* DNA relative to the κ locus and the types of *RS* recombinations that we observed are indicated in Fig. 4*d*.

We believe that the striking similarity to *V–J* joining with the conservation of recognition sequences suggests a biologically important role for *RS* recombinations. This role could simply be a chromosomal deletion mechanism to completely eliminate nonfunctional κ chain genes or to eliminate the enhancer sequences present in the J_κ–C_κ introns of nonfunctional κ genes. Alternatively, the deletion of the chromosomal region containing C_κ may be related to λ chain gene rearrangement. In MOPC315 and CH2, *RS* DNA recombines downstream of a *V–J* joined segment, and in BM18-4.13.9, an *RS* segment recombines directly to a V_κ gene. One could envision a regulatory gene within *RS* DNA that falls under control of the *V*-region Ig promoter after recombination. The products of such a gene might serve as a signal to stimulate λ gene recombination.

If *RS* DNA is of biological importance in the mammalian immune system, one would expect to find a conservation of its recombinational mechanism and overall DNA sequence. Indeed, Heiter *et al.* (2) have reported that in humans the λ chain genes are in germ-line configuration in κ chain-expressing B cells and that in λ chain-producing B cells the κ chain genes are either rearranged or deleted. Thus, in both mice and humans, the deletion of the C_κ regions occurs primarily in cells that have undergone λ gene recombination. A recombined DNA element that recombines into the human J_κ–C_κ region has been isolated from the human B-cell line Nalm-6 (26). This clone is very similar in overall structure to the MOPC315-rs1 and CH2-rs3 clones; all three clones contain a DNA segment that has recombined into the J_κ–C_κ intron replacing the C_κ exon. It will be interesting to determine if this human DNA element has any DNA sequence homology with the mouse *RS* DNA segment.

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