Deletions of Immunoglobulin C_κ Region Characterized by the Circular Excision Products in Mouse Splenocytes

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Summary

We have identified circular DNAs containing the κ light chain constant region (C_κ), as well as the excision products of V_κ-J_κ and V_λ-J_λ joining in adult mouse splenocytes. Analysis of C_κ-positive circular DNA clones revealed two recombination sites (intron recombining sequence [IRS]1 and -2) within the germline J_κ-C_κ intron region and the recombining sequence (RS) located downstream of the C_κ exon. While RS contains a conserved heptamer and nonamer separated by a 23-bp spacer on the 5′ side, IRS1 sequence is an isolated heptamer without an obvious nonamer, and IRS2 contains a variant heptamer, CACAAAA. Since IRS1 and IRS2 recombined with both RS (23-bp spacer signal) and V_κ (12-bp spacer signal) with significant frequency, intron recombination sites seem to have dual recombination signals. These findings provide direct evidence that C_κ deletion preceding λ gene rearrangement can occur by looping out and excision. Increased accessibility of inefficient recombinational loci within the intron may enable recombinase to accept wide signal sequence variation.

Ig proteins are comprised of two identical heavy (H) and two identical light (L) chains. There are two types of Ig L chains, κ and λ, but only a single type is expressed in individual B cells. Mammalian L chain genes become rearranged in an ordered fashion during pre-B cell development such that κ gene recombination precedes λ gene recombination (1). Since many λ chain–producing B cells exhibit deletions of κ chain constant region (C_κ), recombination mediating the loss of the C_κ exon is thought to play a role in the switch from κ to λ rearrangement in maturing B cells (2, 3).

These C_κ deletions use a recombining sequence (RS)[1] located downstream of the C_κ exon. Recombinations of RS DNA involve joining to either a site located in the J_κ-C_κ intron (designated as intron RS or IRS hereafter), or to a V_κ gene (4, 5). While RS DNA is flanked by a 23-bp spacer signal carrying consensus heptamer and nonamer and V_κ by a 12-bp spacer signal, IRS DNA shows an isolated palindromic heptamer without an obvious nonamer. Another site-specific Ig gene recombination mediated by an isolated heptamer has been shown previously in H chain variable gene (V_H) replacement (6, 7). Joining usually occurs only between segments flanked by recognition sequences with unequal spacers (12/23 joining rule) (8, 9), and thus, the recognition sequences dictate which elements can join. Deletional joining of V(D)J recombination generates a reciprocal excision product as a circular DNA molecule according to the 12/23 joining rule in B cells (10–12) and in T cells (13–17). Although nonhomologous recombination of Ig class switching also generates circular excision products (18–20), it remains uncertain whether recombination mediated by an isolated heptamer generates a circular DNA as the reciprocal products.

Since we have cloned excision products of V_L-J_L joining and V_H switch recombination from a highly pure preparation of circular DNA from adult mouse splenocytes (11, 12, 18), we surveyed and identified the recombination products of IRS-RS joining with the appropriate probe from a similar library. We observed that the isolated palindromic heptamer of the IRS recombined with recognition sequences with unequal spacers (V_κ and RS). We also found a novel IRS containing a variant heptamer that recombined with RS. We discuss the violation of the 12/23 joining rule in recombinational events with significant physiological roles and suggest that recombinase is able to accept wide signal sequence variation.

Materials and Methods

Construction of Circular DNA Clone Library. Spleen cells were obtained from five 7-wk-old female BALB/c mice. Circular DNAs were prepared from 2×10^8 cells, and a phage library was prepared by cloning the BamHI fragments of circular DNA into the coliphage Charon 27 λ vector as previously described (12, 18). Phage titers per microgram of vector DNA were 10^5 for self ligation and 3×10^6 for the recombinants.

DNA Hybridization. Plaque hybridization and Southern blot

[1] *Abbreviations used in this paper:* IRS, intron recombining sequence; RS, recombining sequence.

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hybridization were performed according to the methods of Maniatis et al. (21). All DNA probes were used as purified inserts. Probes were as follows: a 2.1-kb HindIII C fragment of λgtWES·IgH701·C (22) for C_μ, a 1.7-kb HindIII-XbaI fragment (23) for J_κ, an 8.6-kb EcoRI fragment of Ig25λ (24) for J_λ, a 280-bp HpaI-AvaII fragment of a Igκ cDNA clone (gift from Dr. T. Honjo, Kyoto University) for C_κ, a 0.9-kb ApaLI fragment of pKDE5 clone (this study) for RS, a 199-bp ApaLI-HindIII fragment of pKDE5 clone for IRS1, a 498-bp SspI fragment of pKDE4 clone (this study) for IRS2, and 11- and 5.2-kb BamHI fragments of rat mitochondrial DNA (25) for mouse mitochondrial DNA.

DNA Sequencing. BamHI-digested circular DNA clones were recloned into a pHSG399 plasmid. Nucleotide sequences were determined by the dideoxy chain termination method (26) using appropriate specific primers synthesized based on the RS 650-bp data base, MUSIGKRS: GACACTGCTCTTTACCCAGT (20-mer; 245–264) for RS: and the J_κ-C_κ 7,466-bp data base, MUSIGKJC2: GAAGCCACAGACATAGACAAC (21-mer; 827–807) for $J_{\kappa1}$; AACAACTTAACAAGGTTAGAC (21-mer; 1184–1164) for $J_{\kappa2}$; CACAAGTTACCCAAACAGAAC (21-mer; 1820–1800) for $J_{\kappa4}$; CTAACATGAAAACCTGTGTC (20-mer; 2155–2136) for $J_{\kappa5}$; AGTCTGTCACATCTCTGTTCT (21-mer; 3255–3235) for IRS1. Nucleotide sequences of V_κ compared are X24 (27), 17-1A (28), V_{139} (29), 61B8 (30), AN02K and AN11K (31), Q52 (32), k2 (33), V_{L38C} (34), V_{ser} (35), V-L7 and V-L6 (36), $V_{\kappa21B}$ (37), f173 and V-L8 (38), V_{1C} (39), and $V_{\kappa24}$ (40).

Results

Circular DNA Clones Containing C_κ Gene Loci. We purified circular DNAs from spleen cells of 7-wk-old BALB/c mice and cloned the fragments after BamHI digestion into the Charon 27 phage vector (cloning capacity up to 9.2 kb). BamHI sites, which are located a short distance downstream of C_κ and upstream of RS (Fig. 1), were expected to be useful for cloning presumptive excision products of C_κ gene rearrangements (2, 4).

We screened circular DNA clones by plaque hybridization with probes of C_κ DNA. Out of 1.2 × 10^6 phage, we obtained 136 C_κ-positive clones (Table 1). The same set of filters was screened with probes of C_H (C_μ) and J_L (J_κ and J_λ). 14 C_μ-positive clones represented the excision products of class switch recombination ongoing in unstimulated spleen cells (18) and 953 J_L-positive clones represented those of V_LJ_L rearrangements performed in the bone marrow (11, 12). Approximately 60% of the phage clones contained mitochondrial DNA.

To characterize the C_κ-positive clones, we selected eight clones at random and compared the restriction fragments (Fig. 1). Four clones contained inserts of 5.7 kb, a size similar to the BamHI fragment expected in the excision product of RS

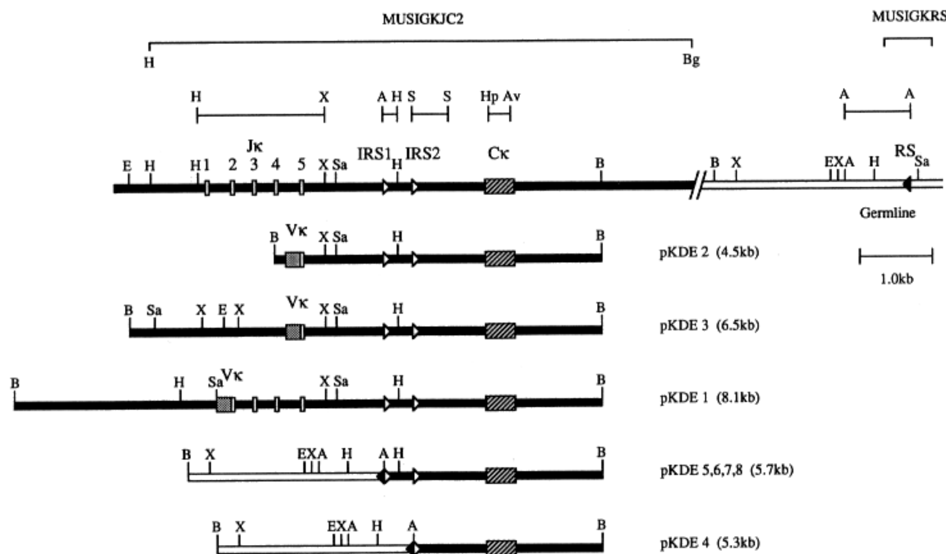

Figure 1. Genomic organization of the J_κ-C_κ-RS loci, and recombinant structures of the C_κ-positive circular DNA clones. DNA probes used (J_κ, IRS1, IRS2, C_κ, and RS) and BamHI fragments of eight clones, pKDE1-8, are compared with the germline restriction map. The areas of genomic DNA sequences (MUSIGKJC2 and MUSIGKRS) registered in the data base are shown at the top. A, ApaLI; Av, AvaII; B, BamHI; Bg, BglII; E, EcoRI; H, HindIII; Hp, HpaI; S, SspI; Sa, SacI; X, XbaI. These sequence data have been submitted to the EMBL/GenBank/DDBJ data libraries under the accession number 57846.

Table 1. *Plaque Hybridization of Circular DNA Clones*

No. of clones screened	No. of clones hybridized with probes:									
				IRS2⁺C_κ⁺RS⁺		IRS2⁺C_κ⁺RS⁻				
	C_μ^+	J_κ^+	J_λ^+	IRS1⁺	IRS1⁻	J_κ^+IRS1⁺	J_κ^-IRS1⁺	J_κ^-IRS1⁻	C_κ^-RS⁺	mt⁺
1.2 × 10^6	14	850	103	74	8	48	4	2	67	ND
200	ND	ND	ND	ND	ND	ND	ND	ND	ND	115

The number of circular DNA clones in the phage libraries characterized by appropriate probes is shown. A set of phage plaque filters was hybridized successively with different probes. Mitochondrial DNA probe-positive (mt⁺) clone is also included.

DNA rearrangement with the J_κ-C_κ intron. Comparison of the C_κ-positive clones with the germline RS locus and J_κ-C_κ intron suggested rearrangements between RS and the J_κ-C_κ intron (pKDE4-8) and between V_κ and J_κ (pKDE1-3). Upon closer inspection of the restriction map, a 5.3-kb (pKDE4) insert was shown to contain RS recombined with a site in the J_κ-C_κ intron, which is more proximal to C_κ than that of the 5.7-kb insert. Clone pKDE4 may represent a new recombination site in J_κ-C_κ intron.

Identification of Recombination Signals of RS/J_κ-C_κ Rearrangements. A synthetic primer downstream of IRS1 sequence (4) reacted with clone pKDE5 but not with pKDE4. Therefore, breakpoints of clones pKDE4 and -5 were sequenced using synthetic primers upstream of RS sequence (650 bp, MUSIGKRS, GenBank; reference 4) and downstream of IRS1 sequence (2), respectively (Fig. 2). Clone pKDE5 contained a precisely fused signal joint of RS with the IRS1 within the J_κ-C_κ intron. Clone pKDE4, however, showed a novel sequence fused with the upstream sequence of RS. By comparison with the published germline J_κ-C_κ sequence (7,466 bp, MUSIGKJC2, GenBank), this novel sequence can be assigned downstream from position 3601. At the junction with RS, a variant signal heptamer, CACAAAA, without an obvious signal nonamer was observed. This is the second signal sequence within the J_κ-C_κ intron, and is therefore termed as the second intron recombining sequence (IRS2), which is distinguished from the known IRS (IRS1). Identity of these two IRS's with the unusual intron recombination points rearranged with V_κ found in myelomas (41) is not clear. To measure the frequency of these two types of recombinants, we prepared two probes, 5'RS (0.9-kb ApaLI fragment) and 3'IRS1 (199-bp ApaLI-HindIII fragment), from clone pKDE5, and a 3'IRS2 probe (498-bp SspI fragment) from clone pKDE4, respectively (Fig. 1). We obtained 82 clones positive for both the C_κ and RS probes. Of these 82 clones, 74 clones were double positive for IRS1 and IRS2 probes and eight were single positive for IRS2 (Table 1). The RS sequence, therefore, recombines with either IRS1 or IRS2 in the J_κ-C_κ intron and deletes the C_κ exon from the chromosome.

Rearrangement Status of $C_\kappa^+RS^-$ Circular DNA Clones. Three clones, pKDE1, -2 and -3, which were suggested to be V_κ/J_κ rearrangements (Fig. 1), contained the germline IRS1 configuration as determined by sequencing with a synthetic primer downstream of IRS1 (Fig. 2). These clones were negative for the RS probe. We obtained 54 clones positive for a C_κ probe but negative for a RS probe from this library. We selected eight clones in addition to clones pKDE1-3. Each insert was shorter than the germline BamHI fragment (12 kb) (42) and was likely to contain rearranged elements (Table 2). The rearrangement status was directly evaluated by reactivity to specific sequence primers downstream of J_κ (Table 2). Every clone except pKDE36 reacted with the primers.

Table 2. *$C_\kappa^+RS^-$ Circular DNA Clones Analyzed*

Clones	Size	Structure of CJ		
		Vκ subfamily	Jκ	Frame
	kb			
pKDE 1	8.1	Vκ4,5 (X24; 100)	Jκ2	–
pKDE 2	4.5	Vκ8 (17-1A; 99.4)	Jκ5	(+)
pKDE 3	6.5	Vκ8 (V139; 100)	Jκ5	–
pKDE 29	5.2	Vκ8 (61B8; 97.3)	Jκ5	–
pKDE 30	4.3	Vκ4,5 (AN02K; 100)	Jκ5	–
pKDE 31	7.2	Vκ1 (Q52; 97.4)	Jκ5	–
pKDE 32	5.3	Vκ12,13 (k2; 84.5)	Jκ2	+
pKDE 33	8.0	Vκ9 (V_{L38C}; 81.9)	Jκ2	–
pKDE 34	4.4	Vκ23 (AN11K; 86.4)	Jκ4	–
pKDE 35	7.2	Vκ1 (Q52; 99.2)	Jκ5	–
pKDE 36	6.6	Vκ28 (Vser; 81.2)	(IRS)	(SJ)

Most homologous V_κ and percent homology are shown in parentheses. +, in frame; (+), in frame with nonsense codon; –, out of frame. Clone pKDE36 has a signal joint (SJ) of V_κ-IRS.

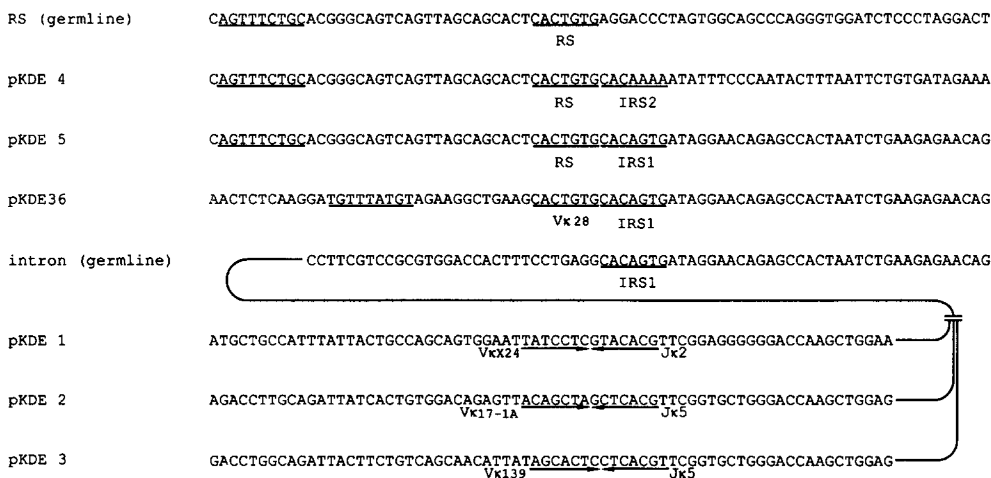

Figure 2. Nucleotide sequences at the recombination sites of circular DNA clones. The recombinant sequences are compared with their corresponding germline sequences of RS (MUSIGKRS) and IRS1. Conflicts between MUSIGKJC2 data base and the IRS1 germline sequence are as follows: bases 3175 (G to C), 3176 (C to G), and 3201 (deletion). The signal sequences are underlined and the coding joints are shown by arrows.

Nucleotide sequencing revealed the V_κ sequence rearranged with J_κ. In Table 2, the most homologous V_κ sequence and the percent homology are summarized for each clone. Most sequences of at least 100-bp nucleotides are assigned to a known V_κ subfamily (43, 44), based on the criterion of 80% homology threshold. Only one translational reading frame (pKDE32) out of 10 coding joints was inphase and free of nonsense codons. Coding joint of pKDE2 was inframe but included a premature termination codon.

Clone pKDE36 did not contain the J_κ region but reacted with the IRS1 probe. Nucleotide sequencing was performed with a specific nucleotide primer downstream of IRS1 (Fig. 2). This clone contained the precise head-to-head fusion of two heptamers in a signal joint resulting from $V_{\kappa 28}$ subfamily recombining with the sequence downstream of IRS1. There is no precedent for such a V_κ/IRS signal joint.

The frequency of these two types of $C_\kappa^+RS^-$ recombinants was measured using a J_κ probe (1.7-kb HindIII-BglII fragment). Of 54 $C_\kappa^+RS^-$ clones, 48 clones were positive for J_κ probe, but six clones were negative (Table 1). Out of six $C_\kappa^+RS^-J_\kappa^-$ clones, four clones were $IRS1^+IRS2^+$ and two clones were $IRS1^-IRS2^+$. Since no obvious signal sequence was found in the flank between IRS1 and IRS2, IRS2 signal sequence is likely to be used to generate the signal joint with V_κ in these two $J_\kappa^-IRS1^-IRS2^+C_\kappa^+RS^-$ clones. These rearranged $C_\kappa^+RS^-$ fragments may represent the final

Table 3. *$C_\kappa^-RS^+$ Circular DNA Clones Analyzed*

Clones	Size	V_κ subfamily used in SJ
	kb	
pKDE 11	6.6	Vκ23 (V-L7; 100)
pKDE 12	6.5	Vκ21 (Vκ21B; 100)
pKDE 13	5.2	Vκ9 (f173; 96.8)
pKDE 14	6.6	Vκ23 (V-L7; 100)
pKDE 15	6.2	VT-1 (V-L6; 89.3)
pKDE 16	4.4	Vκ4,5 (V-L8; 92.8)
pKDE 17	5.1	Vκ9 (f173; 86.3)
pKDE 18	5.2	Vκ1 (V1C; 77.4)
pKDE 19	7.5	Vκ4,5 (V-L8; 90.4)
pKDE 20	7.5	Vκ4,5 (V-L8; 90.4)
pKDE 21	6.0	Vκ1 (V1C; 99.6)
pKDE 22	4.8	VT-1 (V-L6; 80.9)
pKDE 23	6.2	Vκ24 (Vκ24; 71.2)
pKDE 24	10.0	Vκ4,5 (V-L8; 88.5)
pKDE 25	6.5	Vκ21 (Vκ21B; 100)
pKDE 26	4.8	Vκ28 (Vser; 82.9)
pKDE 27	5.0	Vκ28 (Vser; 82.7)
pKDE 28	5.3	Vκ12,13 (k2; 78.0)

Most homologous V_κ and percent homology are shown in parentheses.

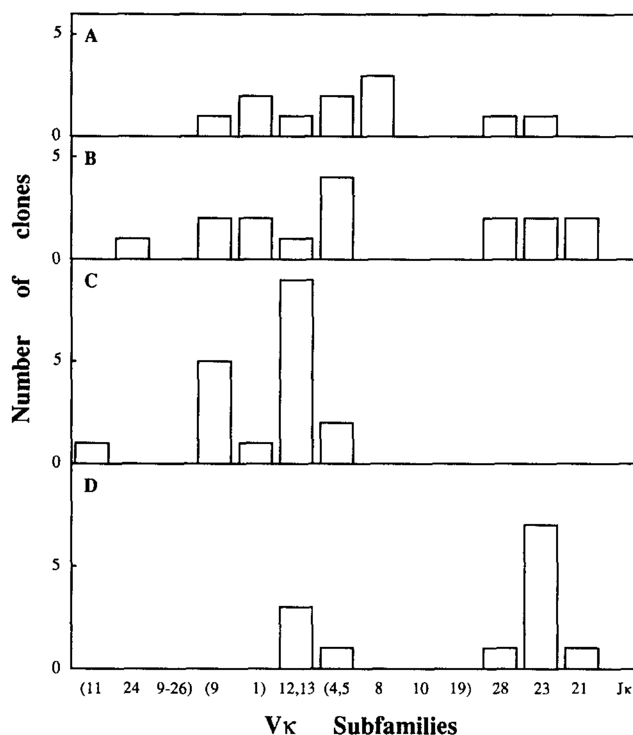

Figure 3. Distributions of rearranged V_κ gene segments found in circular DNA clones. Relative germline positions of the V_κ gene subfamilies are shown along the *x*-axis (43, 44); the order of families within parentheses is not decisive. (*A*) V_κ rearrangements of $C_\kappa^+RS^-$ circular DNA clones (Table 2). (*B*) V_κ rearrangements of $C_\kappa^-RS^+$ circular DNA clones (Table 3). (*C* and *D*) V_κ's used in a coding joint (*C*) or a signal joint (*D*) of circular double recombination products generated by V_κ-J_κ rearrangements (11, 12).

rearrangement status of alleles that proceed to C_κ-deleting secondary rearrangements.

$RS^+C_\kappa^-$ Circular DNA Clones and RS/V Rearrangements. We obtained 67 clones positive for a RS probe but negative for a C_κ probe from the same library (Table 1). To evaluate the rearrangement status, 18 clones were selected and sequenced using a synthetic primer upstream of RS. Each clone showed a signal joint composed of precisely fused heptamers in a head-to-head fashion. The downstream sequence from the signal joint was assigned to a member of the V_κ subfamilies (43, 44). The results were summarized in Table 3. These clones represent the excision products of V_κ/RS rearrangements resulting in the C_κ gene deletion.

Discussion

Many λ chain–producing B cells exhibit deletion of the C_κ region and, hence, the C_κ-deleting recombination is suggested to have a role in the initiation of λ gene rearrangement during B cell maturation (2, 4). It has been shown that the C_κ deletion is due to the recombination of RS DNA located downstream of the C_κ exon. Recombinations of RS DNA involved joining to either a site within the J_κ-C_κ intron or to a V_κ gene (2, 4). In a circular DNA library from

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Consensus V                   CACAGTG--12bp--ACAAAAACC

                              *******        *********
Vκ21C                         CACAGTG--11bp--ACAAAAACC

                              *******         **  ** *     *  * ***
IRS1                          CACAGTG--12bp--CCACTAATC-2bp-AAGAGAACA

                              ****           **   **          * *
IRS2                          CACAAAA--12bp--ACTTTAATT-2bp-GTGATAGAA

               *  **          *******        *********
IgH Dsp2      TATGGT(T/A)-5bp-CACAGTG--12bp--ACAAAAACC

                              *******                       ** *****
δRec1                         CACAGTG---------23bp---------GCATAAACC

                              *****                        * * **
δRec2                         CACAGAC---------23bp---------ATATAAGAA

                              *** **                       ****
δRec3                         CACTGTA---------23bp---------CCAAATCTA


Consensus J                   GGTTTTTGT---------23bp---------CACTGTG

                              *********                      *******
Jκ1                           GGTTTTTGT---------23bp---------CACTGTG

                               **** **                       *******
RS                            AGTTTCTGC---------23bp---------CACTGTG

                                            *********          *****
ψJα                                         GGTTTTTGT--12bp--TTCTGTG

                                                              ******
VH internal                                                  TACTGTG
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Figure 4. Comparison of signal sequences. Consensus V and J sequences (8); V_κ21C and J_κ1 (23); IgH Dsp2 (49); δRec's and ψJα (17); V_H internal heptamer (6, 7); and other sequences from this paper. More or less essential nucleotides in the consensus sequences are underlined (51). The bases found in a consensus sequence are marked by an asterisk.

adult mouse splenocytes, we identified circular DNA excised by the recombination of RS DNA and obtained 82 $C_\kappa^+RS^+$ clones and 67 $C_\kappa^-RS^+$ clones, representing RS/J_κ-C_κ and RS/V_κ rearrangement, respectively. From the same library, we also obtained an equivalent amount of 103 $J_{\lambda1}^+$ clones that represent the excision products of V_λ-$J_{\lambda1}$ rearrangements. The coincidence in frequency of excision products by RS recombination and V_λ-J_λ recombination is consistent with the notion of a regulatory role for C_κ deletion in the initiation of λ gene rearrangement.

According to the studies on λ and κ gene rearrangements in pre-B cell differentiation (45), RS rearrangement occurs mainly toward the end of the κ-recombining phase of pre-B cell development, before the initiation of λ recombination. Therefore, the rearrangement status of V_κ and J_κ found in the excision products of C_κ-deleting recombination may represent the terminal phase of κ rearrangements before κ to λ isotype switching. We found that the $J_{\kappa5}$ segment located at the 3′ end of the J_κ cluster is most frequently used (Table 2), in contrast to the biased usage of $J_{\kappa1}$ at the most 5′ side observed in the excision products of primary V_κ-J_κ rearrangement (12). This suggests that $J_{\kappa5}$ is the last J_κ used after the multiple rounds of V_κ-J_κ recombinations.

The pattern of V_κ usage in the excision products of C_κ was compared with a tentative genetic map of the V_κ locus (43, 44). The distributions of used V_κ are shown in Fig. 3 along with the summarized data of V_κ usage in the excision products of V_κ-J_κ rearrangements (11, 12). The V_κ usage in the terminal phase of κ rearrangements were dispersed throughout the locus (Fig. 3, *A* and *B*), in contrast to the biased usage of J_κ-distal V_κ in primary rearrangement and of J_κ-proximal V_κ in secondary deletional events (Fig. 3, *C* and *D*). Since approximately half of the V_κ gene segments lie in the opposite polarity on the chromosome relative to the J_κ segments (46), primary inversion events may engage distal V_κ gene segments while preserving proximal genes for secondary rearrangements by deletion. However, at the terminal phase of κ rearrangements after multiple rounds of inversion and deletion, the original chromosomal position of V_κ may have been shuffled. Usage of dispersed V_κ in the early and late rearrangements of genes has been shown by others (47, 48) and is in contrast to the biased usage of J_H-proximal V_H in VDJ rearrangements (1).

While we have shown that the productive V_κ-J_κ rearrangements occur in approximately one out of every three rearrangements (12), only 1 out of 10 V_κ-J_κ coding sequences excised by the RS recombination showed a productive κ gene rearrangement (Table 2). Upon closer inspection of rearrangement status, all V_κ's rearranged with $J_{\kappa5}$ were nonproductive, but V_κ's rearranged with the other J_κ's (which can serve as a substrate for further rearrangement) showed one productive rearrangement of four coding joints. This agrees with previous data showing a lack of feedback inhibition by functional V_κ gene rearrangement (12). These results suggest that premature B cells that have generated both intermediate and dead-end products in stochastic mechanisms may switch to λ gene rearrangements by RS recombination.

Recognition sequences that mediate site-specific recombination flank all Ig gene segments involved in V(D)J gene rearrangement. A complete recognition sequence contains a highly conserved palindromic heptamer (head), which is contiguous to the end of the coding sequence, and a characteristic nonamer (tail), separated from the heptamer by a nonconserved spacer region of either 12 or 23 bp. Joining appears to occur only between elements flanked, respectively, by recognition sequences having spacers of 12 and 23 bp (12/23 joining rule) (8, 9). In Fig. 4, we summarize the recognition sequences involved in the RS recombination. While the RS sequence contains complete recognition signals, IRS1 and IRS2

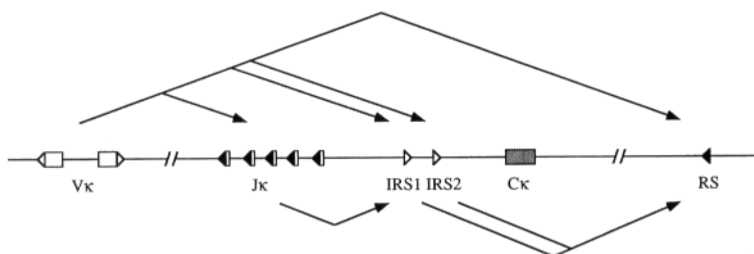

Figure 5. Multiple recombination pathways resulting in the inactivation and deletion of C_κ exon. Arrow shows the direction of donor to acceptor signal in deletional recombination by normal or pseudonormal joining. Inversional recombinations are also possible for V_κ's in the opposite polarity. Recombination signal sequences and the polarity are shown as a filled triangle for a 23-bp spacer signal, an open triangle with the rectangle for a 12-bp spacer signal, a single open triangle for an isolated heptamer, and rectangles for V, J, C exons.

sequences seem to be isolated heptamers without obvious nonamer sequences. Other isolated recognition heptamers with physiological roles are found in the 3'-internal region of most V_H genes used for V_H gene replacements (6, 7). IRS1 recombined with either a 23-bp spacer signal (RS) or a 12-bp spacer signal (V_κ), forming head-to-head fused heptamers. The palindromic nature of the heptamer would suggest the possibility of either direct or inverted signal alignment in an intermediate structure. However, signal heptamers of IRS may have been polarized as head to tail, thereby preventing C_κ inversion mediated by IRS/RS rearrangements. Alternatively, the IRS heptamer may be provided by a binary spacer signal. Weak nonamer signal motifs are recognizable at either 12- or 23-bp distance downstream of the signal heptamer (Fig. 4). Other binary spacer signals containing an additional heptamer have been implied in the IgH D_{SP2} fragment (49, 50).

According to Hesse et al. (51), the first three bases (CAC) closest to the head in the consensus heptamer sequence are strictly required, and the middle base, A, is less stringently specified, while the sixth and seventh positions (AA) of the nonamer may be necessary for efficient recombination. In this context, IRS1 and IRS2 contain similar signal sequence motifs. Less recombination efficiency of IRS2 with the RS compared with the IRS1/RS recombinations is possibly due to the variant heptamer (CACAAAA) (Fig. 4). A deletional rearrangement analogous to the IRS/RS recombination is the joining of TCR δ gene deleting elements (δRec's) with ψJ_α flanking the C_δ gene, leading to C_δ exon deletion (17, 52). While the δRec1 sequence contained a complete recombination signal, δRec2 and -3 sequences seem to be less efficient due to lack of the necessary requirements.

Although the presence of dual recognition signals requires modification of the 12/23-bp joining rule, all recombinations analyzed in splenocytes contained standard recombination products (coding joints and signal joints) with no unusual products; neither hybrid joints nor open-and-shut joints (53) were observed. The prevalence of nonstandard recombination for extrachromosomal substrates may not accurately reflect rearrangement in the endogenous context.

There are several pathways to delete or inactivate the κ genes before the initiation of λ gene rearrangement as summarized in Fig. 5. The RS/IRS recombination results in a deletion of the C_κ region, whereas the RS/V_κ recombination deletes the entire J_κ-C_κ region. These recombinations result in the elimination of the κ enhancer elements located on both sides of C_κ (54). Thus, C_κ deletion could prevent the useless expression of nonfunctional κ genes in λ chain–producing cells and ensure λ gene use. Selsing et al. (55) have proposed that the RS recombinants could encode a *trans*-acting factor that signals the activation of the λ genes for recombination. We provided direct evidence of C_κ excision products generated by the standard recombination in both pathways of RS/IRS and RS/V_κ joining. We also provided evidence for standard IRS-V_κ recombination products mediated by either inversional or pseudonormal joining depending on the orientation of V_κ. Recently, a novel two-step rearrangement pathway, in which J_κ/IRS1 rearrangement forms a signal joint capable of further V_κ recombination, has been shown to occur with significant frequency in the Abelson MuLV–transformed cell line (42). These standard rearrangements of IRS with J_κ or V_κ effectively exclude functional expression from that allele. Thus, IRS plays a key role for both recombination pathways resulting in C_κ deletion and the cessation of nonfunctional κ gene expression. An unusual recombination event for the V_κ-IRS hybrid joint and pseudonormal J_κ-IRS joining has been found in myelomas (56) and plasmacytomas (57), respectively. Although the J_κ-C_κ intron is transcriptionally active after multiple V_κ-J_κ rearrangements, the destructive recombinational activity of IRS loci remains limited, perhaps due to the weak recombination signal motifs. Activation of the IRS loci for recombination resulting in C_κ deletion may occur as part of the developmental switch that leads to λ chain rearrangement.

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