Lack of Feedback Inhibition of Vκ Gene Rearrangement by Productively Rearranged Alleles

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Summary

Circular DNAs excised by immunoglobulin κ chain gene rearrangements were cloned and characterized. 16 of 17 clones examined were double recombination products containing a Vκ-Jκ rearrangement (coding joint) as well as the reciprocal element (signal joint) of another Vκ-Jκ rearrangement. These products suggested multiple recombination, primary inversion, and secondary excision. In primary events, 5 of 16 translational reading frames were in-phase. Thus, Vκ gene rearrangement may not be inhibited by the presence of a productively rearranged allele. An unusually large trinucleotide (P) insertion forming a palindrome of 12 nucleotides was also observed in one of the coding joints.

In B cells, feedback inhibition of immunoglobulin heavy chain (IgH) gene rearrangement is believed to be due to the expression of membrane-bound μ chains (1–3), whereas the cessation of Vκ gene rearrangement is thought to be inhibited by the combination of IgH chain and light chain (IgL) gene products (3–6). Nevertheless, a transgenic κ chain present in the cytoplasm did not shut off the endogenous κ gene rearrangement (5). It is also reported that corrective V-J recombinations, with displacement of the nonproductive κ gene, occur with a significant frequency in clonal cell lines (7). This suggests that secondary recombinations of one allele may continue unless prevented by the feedback inhibition of a functional product. Two independent B cell lineages that differ in response to feedback inhibition by the membrane bound immunoglobulin are also postulated (6). Thus, it is currently unclear whether or not L chain allelic exclusion mechanisms are operative.

Recently, we characterized the circular DNA generated by inversional and excisional Vκ-Jκ joining and stored in adult mouse splenocytes, thereby providing evidence of multiple recombination events occurring at the Igκ locus (8). These circular double recombination products from a single κ chain allele allow us to examine whether or not primary inversional recombinations are productive.

In this study, we show that the translation reading frames of 5 of 16 primary recombinants examined are in-phase, suggesting that exclusion of Vκ gene rearrangements by a functional allele may involve utilization of successive Vκ products.

Materials and Methods

Preparation of Circular DNA Clone Library and Plaque Hybridization. Circular DNAs were prepared from splenocytes of 8-wk-old mice (BALB/c-*nu/nu*) and purified by use of ATP-dependent DNase according to the method described previously (9). Since no single-stranded DNA fragments were found by electron microscopy of fractions of covalently closed circular DNA of splenocytes obtained by the CsCl-EtBr buoyant density method, nitrocellulose column chromatography before the ATP-dependent DNase treatment was not required. This is in contrast with preparations from thymocytes that contain a large amount of single-stranded DNA fragments that are inhibitory to the enzyme action on double-stranded DNA. Digestion of linear DNA fragments was almost complete, and the purity was >96% obtained with the circular DNA fraction of thymocytes.

Purified circular DNAs were digested by EcoRI and cloned into λgt11 phage vector as described (10). Recombinant phage titer of EcoRI-digested vector DNA was $1.8 \times 10^6/\mu g$. Plaque hybridizations were performed with DNA probes of Jκ (1.7-kb HindIII/XbaI fragment) (11). We have obtained 18 $J\kappa^+$ clones from 2.0×10^5 phages.

DNA Sequence Analysis. 17 $J\kappa^+$ clones were recloned into pHSG399. Signal joints and coding joints of V-J joining in each clone were sequenced by the specific primer-directed chain-termination method (12) using synthetic primers upstream of J (GTTAAGCTTTCGCCTACCCAC for Jκ1, TTACTCGGTGCTCAGACCAT for Jκ2, AGGGATAATTGTCTACCTAGG for Jκ3, GCCTATCTAACTGGATCGCCT for Jκ4, TCCTCTGAATTTGGCCCATCT for Jκ5); and downstream of J (GAAGCCACAGACATAGACAAC for Jκ1, AACAACTTAACAAGGTTAGAC for Jκ2, CACAAGTTACCCAAACAGAAC for Jκ4) (11). Nucleotide sequences used as references are M41 (13), A25.9.7 cDNA (14), S107A cDNA (15), 70Z/3 cDNA (16), K2 (17), TF2-36 cDNA (18), Vκx36 (8), L6, L7 (19), VκSer (20), Vκ21-C (21), L8 (22), rat κ chain IR162 cDNA (23), and Jκ germline sequences (11).

Results and Discussion

All the inserts of $J\kappa^+$ clones were different from the germline EcoRI fragment (15 kb) and were therefore likely

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Table 1. *Circular DNA Clones Characterized*

Clones	Size	Vκ gene subfamily used in:		Jκ used in:		CJ* frame
		Coding joint (CJ)	Signal joint (SJ)	CJ	SJ	
	kb					
MSI-N101	4.5	$V\kappa_9$(M41;100)‡	$V\kappa x^S$(X36;99)	Jκ1	Jκ5	–
MSI-N102	3.2	$V\kappa_9$(M41;100)	Vκx(X36;99)	Jκ4	Jκ5	+
MSI-N103	3.2	$V\kappa_{12,13}$(A25.9.7;99)	$V\kappa_{23}$(L7;100)‖	Jκ1	Jκ2	–
MSI-N104	5.9	$V\kappa_{22}$(S107A;99)	$V\kappa_{28}$(V-Ser;99)	Jκ4	Jκ5	–
MSI-N105	4.2	$V\kappa_9$(M41;100)	Vκx(X36;99)	Jκ1	Jκ4	–
MSI-N106	7.2	$V\kappa_{4,5}$(70Z/3;93)	$V\kappa_{12,13}$(K2;94)	Jκ1	Jκ4	–
MSI-N108	3.7	–	$V\kappa_{21}$(21C;100)	–	Jκ2	–
MSI-N109	3.6	$V\kappa_{12,13}$(K2;100)	$V\kappa_{12,13}$(K2;91)	Jκ4	Jκ5	–
MSI-N110	3.9	$V\kappa_{12,13}$(A25.9.7;99)	$V\kappa_{23}$(L7;100)‖	Jκ2	Jκ5	–
MSI-N111	3.5	$V\kappa_{12,13}$(A25.9.7;99)	$V\kappa_{12,13}$(K2;98)	Jκ1	Jκ2	+
MSI-N112	4.5	$V\kappa_9$(M41;100)	Vκx(X36;99)	Jκ1	Jκ5	–
MSI-N113	3.2	$V\kappa_{12,13}$(A25.9.7;99)	$V\kappa_{23}$(L7;100)‖	Jκ1	Jκ2	–
MSI-N114	5.2	$V\kappa_{4,5}$(70Z/3;91)	$V\kappa_{4,5}$(L8;78)¶	Jκ1	Jκ4	–
MSI-N115	4.3	$V\kappa y^S$(IR162;80)**	V_{T-1}(L6;80)‡‡	Jκ1	Jκ5	+
MSI-N116	3.2	$V\kappa_{12,13}$(A25.9.7;99)	$V\kappa_{23}$(L7;100)‖	Jκ1	Jκ2	+
MSI-N117	4.4	$V\kappa_{12,13}$(A25.9.7;99)	$V\kappa_{23}$(L7;100)‖	Jκ1	Jκ5	+
MSI-N118	6.7	$V\kappa_1$(TF2-36;99)	V_{T-1}(L6;100)	Jκ2	Jκ5	–

* In frame (+) or out-of-frame (–).
‡ Most homologous Vκ and percent homology in parentheses.
§ Vκx and Vκy; Vκ unassigned to known subfamily.
‖ Homology of 43 bp downstream from SJ.
¶ Homology of 100 bp downstream from SJ.
** Homology of 200 bp except downstream 9 bp.
‡‡ Homology of 55 bp downstream from SJ.

to contain rearranged elements (Table 1). Each $J\kappa^+$ clone, except clone MSI-N108, contained two recombination sites of VJ joining; a coding joint (CJ)[1] and a signal joint (SJ). The presence of two recombinant structures in a single clone represents successive Vκ to Jκ joining events. Since clones containing a single recombinant structure of a signal joint are rare, such excision products may have been diluted out during cell division. Alternatively, initial V-J joining may preferentially involve inversions rather than deletions.

The sequences of the 170–290-bp nucleotides upstream or downstream from the recombination sites in each clone revealed the precise head-to-head fusion of two heptamers in the signal joint and Vκ sequences utilized in the Vκ-Jκ joinings. Identification of the most homologous Vκ sequence and the percent homology are summarized for each clone in Table 1. Most sequences are assigned to a known Vκ subfamily (24), based on the criterion of 80% homology threshold. Identical Vκ coding sequences are shared by clones MSI-N101, N102, N105, N112($V\kappa_9$); MSI-N103, N110, N111, N113, N116, N117($V\kappa_{12, 13}$); and MSI-N106, N114($V\kappa_{4, 5}$). Identical Vκ sequences downstream from the signal joint are also shared by clones MSI-N101, N102, N105, N112(Vκx); and MSI-N103, N110, N113, N116, N117($V\kappa_{23}$). However, every clone is generated by independent recombinational events as determined by the junctional diversity. Four clones 99% homologous with the Vκx36(8) and a clone MSI-N115 may represent unknown Vκ subfamilies since no homologies were found in the published mouse Vκ sequences. However, clone MSI-N115 showed 80% sequence similarity with rat Vκ gene IR162 (23).

We found that the Jκ1 segment located at the most 5′ side of the Jκ cluster is more frequently utilized at the primary rearrangement (CJ) than the Jκ2 and Jκ4 segments (Table 1). The Jκ3 segment was not detected in any rearrangement, probably because of the base substitution at the signal heptamer. The Jκ5 segment was not found in any primary recombination structures. This biased primary rearrangement of Jκ1 may be compensated by secondary rearrangements since previous reports indicate that both Jκ1 and Jκ2 segments are used for V-J rearrangement and transcription more frequently than were the Jκ4 and Jκ5 segments (25, 26).

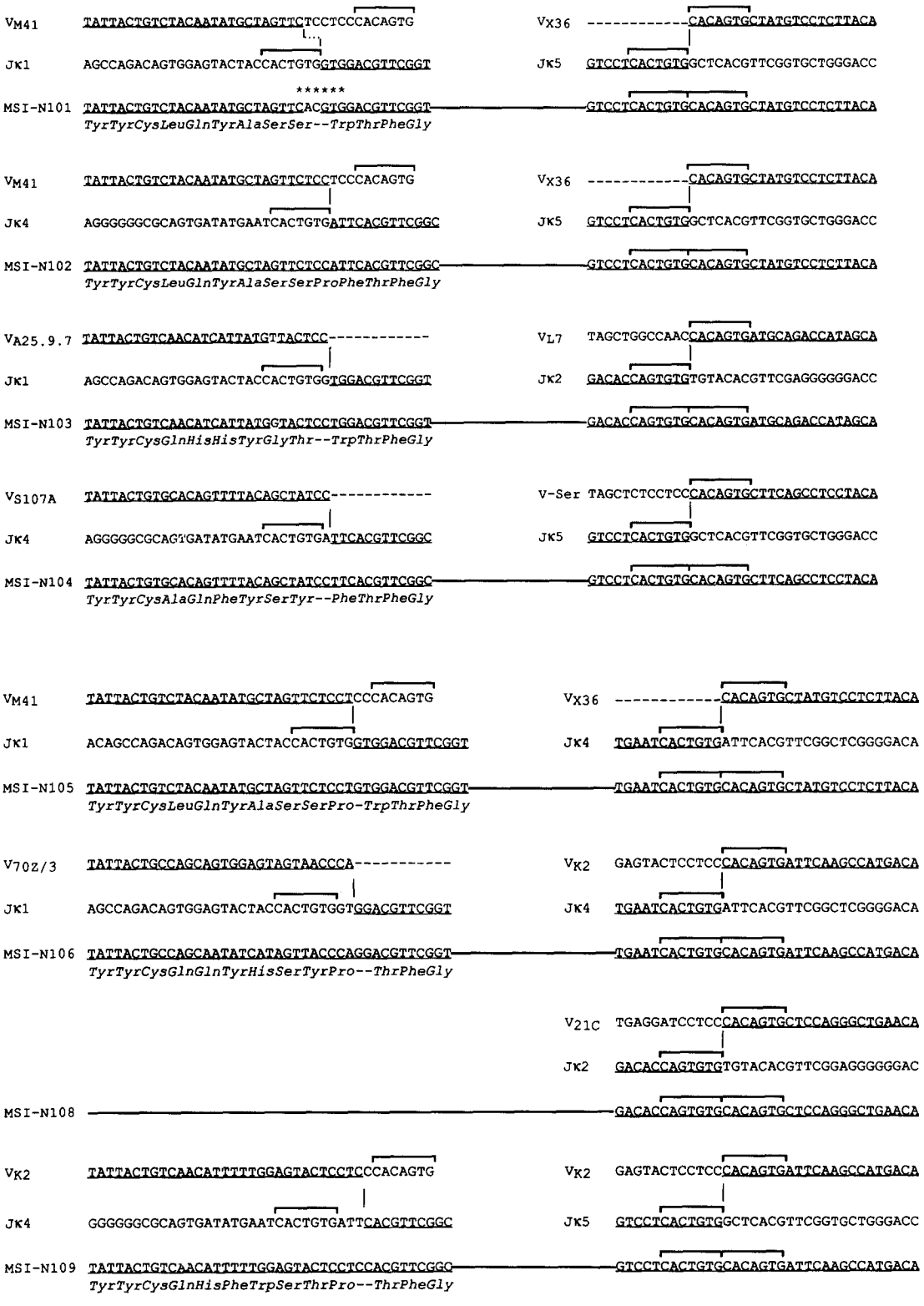
[1] *Abbreviations used in this paper:* CJ, coding joint; SJ, signal joint.

The 100–300 Vκ elements, spanning an estimated 500–2,000 kb of DNA, are organized into 18 subfamilies with at least 40% of the Vκ genes in an opposite transcriptional orientation relative to the Jκ locus (24, 27, 28). These subfamilies are suggested to be a continuum of related sequences (29). Relative positions of Vκ and Jκ subfamilies are tentatively mapped by recombinant inbred strain analyses as follows: centromere; (V_{11}, V_{24}, $V_{9\text{-}26}$); (V_9, V_1), $V_{12,13}$; (V_4, V_8, V_{10}, V_{19}); V_{L8}; V_{23}; V_{21}; Jκ1–5; Cκ (24, 28). Recombination of Vκ genes in the same transcriptional orientation as Jκ will delete the intervening DNA, forming a circular DNA, whereas recombination of those in the opposite transcriptional orientation will invert the intervening DNA bringing germline distal Vκ genes closer to Jκ. Since there is no strongly preferred site orientation in these excisive or inversional recombinations (30), primary recombination products retained on chromosome are positioned to be excised by secondary rearrangements. We have evaluated the primary recombinations of the circular clones by noting the relative germline positions of Vκs utilized in both CJ and SJ recombinations. Five clones, MSI-N103, N110, N113, N116, and N117, utilized Jκ-distal $Vκ_{12,13}$ segments in the primary (CJ) event, and Jκ-proximal $Vκ_{23}$ segments in the secondary (SJ) event, showing successive inversion and deletion events. Another five clones, MSI-N101, N102, N105, N112, and N118, utilized $Vκ_9$ and $Vκ_1$ subfamilies, which are relatively distal to Jκ, in the coding joints, although the Vκs in the signal joint have not been mapped. All four clones examined in the previous study have suggested that excision of circular DNA was preceded by inversion (8). Only clone MSI-N106 represents successive deletional events generating CJ with Jκ-proximal $Vκ_{4,5}$ and SJ with Jκ-distal $Vκ_{12,13}$. Although the Vκ genes lacking EcoRI site in the 3′ flank may not be cloned in the excision products, rare primary excision products having a single signal joint are consistent with the preferential inversional recombination in the primary event. Vκ gene clusters in the same transcriptional orientation may be favored by recombinase at the level of substrate accessibility due to open chromatin (31). For successive rearrangements, Vκ gene clusters inverted in the first event are necessarily more likely to be excised in the second event (8). Our data (Table 1) support the conclusion that Vκ usage is distributed throughout the locus and different from biased utilization of the most J_H-proximal V_H gene segments (32–34).

Junctional sequences of circular DNA clones are shown in Fig. 1 and compared with the corresponding V or J segments. Some nucleotides are removed from the coding sequence of Vκ or both Vκ and Jκ before forming a coding joint. For the 5′ terminals of intact Jκ sequence, insertion of P nucleotides (35) forming a palindrome with the 5′-terminal nucleotides of Jκ is seen in the coding joint of clones MSI-N101 and -N112. We also found a long palindrome of 12 bp in the coding joint of MSI-N113. Insertion of trinucleotides (GGA) may represent a part of P nucleotides flipped from the other strand of the 3′-terminal hexanucleotides of $Vκ_2$ (TCCTCC). There is no precedent for trinucleotide P insertion composing a 12-bp palindrome. We have previously seen a 10-bp palindrome in the Vκ-Jκ coding joint on excision products, which was possibly formed by the flip-flop of the other strand of 3′-terminal pentanucleotides of Jκ1 (8). In place of an addition of N nucleotides by terminal transferase, P nucleotides seem to contribute to the diversification of coding joints in κ chain rearrangements.

No Vκ genes homologous to the V-J coding sequence on circular DNA were pseudogenes. Moreover, five translational reading frames (MSI-N102, -N111, -N115, -N116, -N117) out of 16 coding joints were in-phase and free of nonsense codons. These productive rearrangements occur in approximately one out of every three rearrangements, as expected in genomic V gene assembly. Nevertheless, these genes are deleted by the secondary rearrangements. Seemingly, there is no feedback inhibition of secondary rearrangements by the generation of a productive CJ. Identification of an in-phase V-J structure in the circular DNA clones was unexpected, since it has been shown that corrective Vκ-Jκ recombinations, with displacement of a nonproductive κ gene, occur with significant frequency in developing transformed pre-B cells (7).

There are four possible explanations for the displacement of in-phase V-J structures resulting in circular DNA. First, these in-phase V-J structures could contain nonfunctional genes, due to somatic mutations in transcriptional regulatory elements. Second, the CJ may be formed on the circular DNA after excision from the chromosome. Concomitantly in this case, circular DNA molecule having a single SJ should be generated as the reciprocal product. However, such single SJ structure clones were very rare in κ chain circular DNA libraries (Table 1). Moreover, at least five (and perhaps more) of our clones contain: (*a*) a coding joint derived from a distal V; and (*b*) a SJ derived from a proximal V and downstream J. This cannot represent an excision or inversion event on a pre-existing circular molecule. We conclude that such clones represent a primary inversion event on the chromosome, followed by replacement of the V-J by deletional rearrangement of a second V, thereby generating a circle with two joints. Third, the CJ may be retained on the circular DNA by excising the segment between an upstream Vκ and the downstream previously inverted Jκ oriented in the opposite polarity (pseudo-normal joining) (27, 36). However, inversion of clustered Jκs is not expected since our data indicate a preferential primary inversion of the most 5′ side of the Jκ cluster. The fourth explanation is that the productive rearrangements generate cytoplasmic κ chains that cannot pair effectively with the pre-existing cellular H chains to make complete immunoglobulin molecules capable of turning off L chain gene rearrangement. The allelic exclusion of the endogenous κ gene by a κ transgene was observed only when combinations of κ and H chains were present (6). Here, we propose that various L chain alleles are sequentially rearranged and that products of in-phase joints are tested for the best functional interaction with the pre-existing H chain in the cell.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TyrTyrCysLeuGlnTyrAlaSerSer--TrpThrPheGly
VX36 ----------CACAGTGCTATGTCCTCTTACA
JK5 GTCCTCACTGTGGCTCACGTTCGGTGCTGGGACC
GTCCTCACTGTGCACAGTGCTATGTCCTCTTACA
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JK4 AGGGGGGCGCAGTGATATGAATCACTGTGATTCACGTTCGGC
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TyrTyrCysLeuGlnTyrAlaSerSerProPheThrPheGly
VX36 ----------CACAGTGCTATGTCCTCTTACA
JK5 GTCCTCACTGTGGCTCACGTTCGGTGCTGGGACC
GTCCTCACTGTGCACAGTGCTATGTCCTCTTACA
VA25.9.7 TATTACTGTCAACATCATTATGTTACTCC----------
JK1 AGCCAGACAGTGGAGTACTACCACTGTGGTGGACGTTCGGT
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TyrTyrCysGlnHisHisTyrGlyThr--TrpThrPheGly
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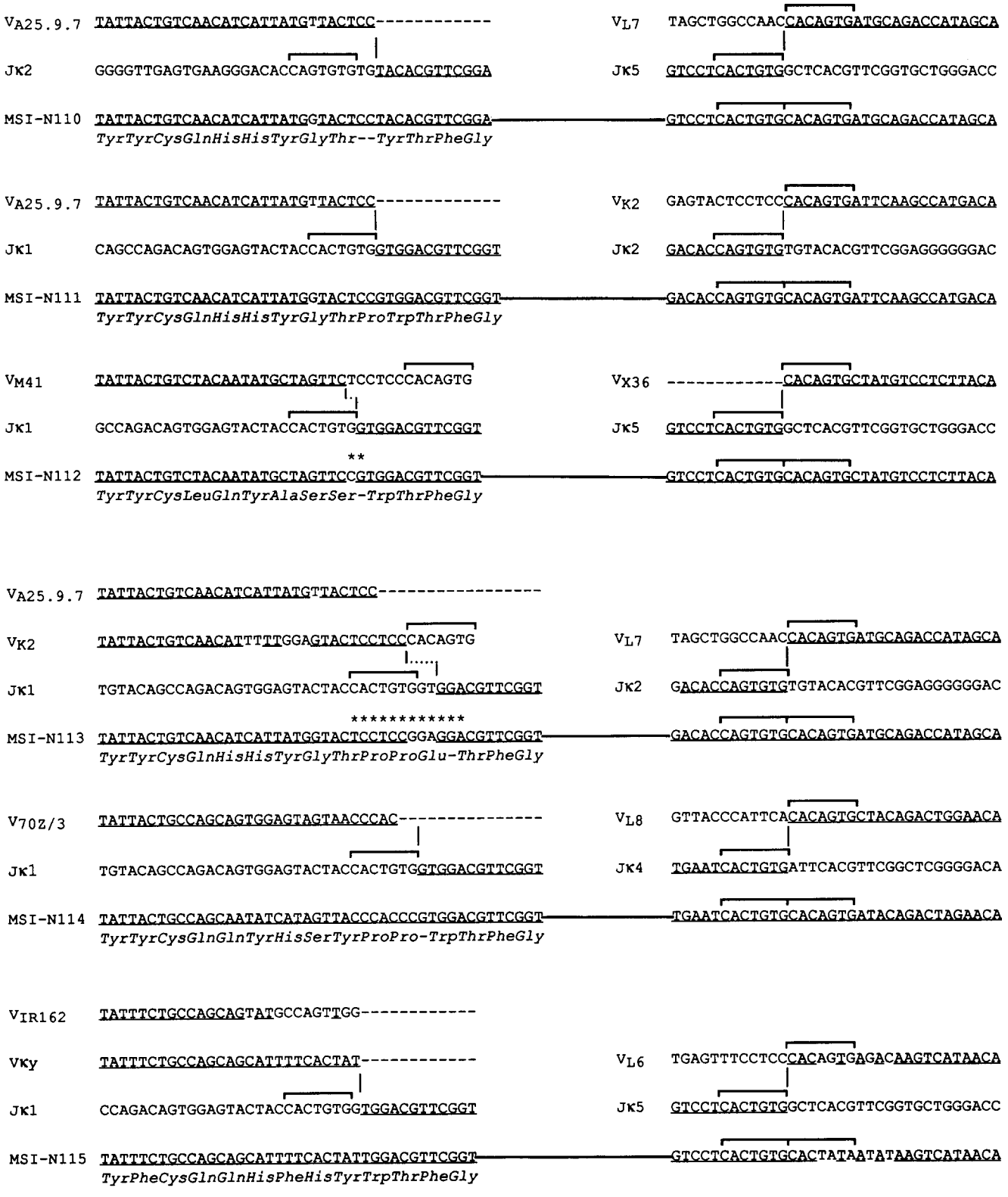
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TyrTyrCysGlnHisHisTyrGlyThr--TyrThrPheGly
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VM41 TATTACTGTCTACAATATGCTAGTTCTCCTCCCACAGTG
VX36 ----------CACAGTGCTATGTCCTCTTACA
JK1 GCCAGACAGTGGAGTACTACCACTGTGGTGGACGTTCGGT
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TyrTyrCysLeuGlnTyrAlaSerSer-TrpThrPheGly
VA25.9.7 TATTACTGTCAACATCATTATGTTACTCC----------
VK2 TATTACTGTCAACATTTTTGGAGTACTCCTCCCACAGTG
VL7 TAGCTGGCCAACCACAGTGATGCAGACCATAGCA
JK1 TGTACAGCCAGACAGTGGAGTACTACCACTGTGGTGGACGTTCGGT
JK2 GACACCAGTGTGTGTACACGTTCGGAGGGGGGAC
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V70Z/3 TATTACTGCCAGCAGTGGAGTAGTAACCCAC----------
VL8 GTTACCCATTCACACAGTGCTACAGACTGGAACA
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VIR162 TATTTCTGCCAGCAGTATGCCAGTTGG----------
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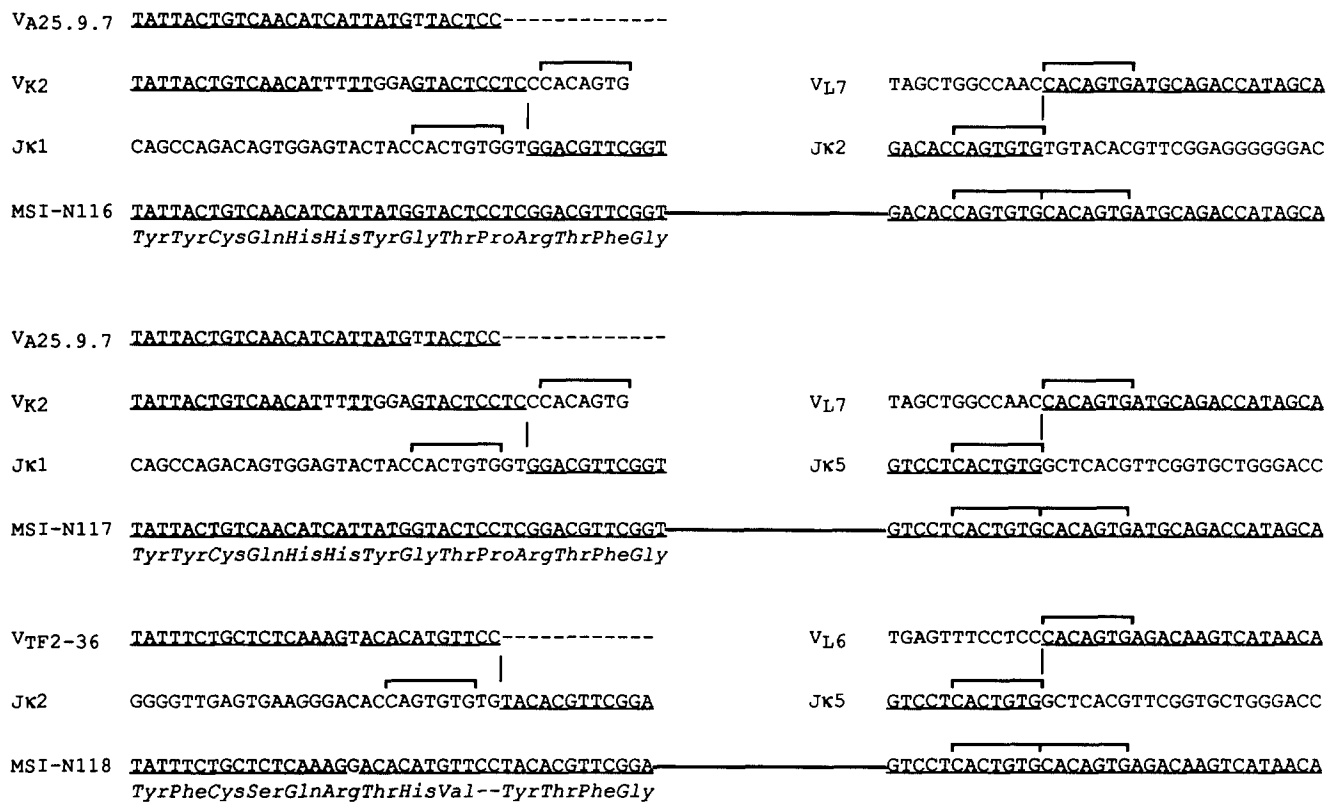

Figure 1. Nucleotide sequences at the coding joint (*left column*) and the signal joint (*right column*) of circular DNA clones. The recombinant sequences are compared with the most homologous V or J sequences. The homologous sequences are underlined and the breakpoint is connected by a vertical line. Signal heptamers are bracketed. Two recombination sites on the same circular DNA clone are linked by a solid line. Nucleotides forming a long palindromic structure are shown by asterisk. Amino acid framework is shown by three letters. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers 54753–54771.

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