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Mapping of immunoglobulin variable region genes: relationship to the 'deletion' model of immunoglobulin gene rearrangement

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ABSTRACT

Five families of variable region genes of mouse kappa chains were analyzed by Southern blot hybridization to determine their relative chromosomal map positions. Map positions were deduced by V_K gene deletion from antibody-producing cells expressing upstream V_K genes and retention in cells expressing downstream genes. The V_K regions expressed in the myelomas MOPC167, MPC11, MOPC21 and ABPC20 are members of V_K families exhibiting one, three, six and six major germline hybridization bands respectively. The gene order of the five families in germline DNA was found to be V_{M167} - V_{M11} - (V_{M21}, V_{A20}) - V_{ABE8} - J_K - C_K. As expected in a deletion model of immunoglobulin gene rearrangement, a sequence located just 5' of J_1 in germline DNA was found to be absent from some antibody producing cells which had not retained any germline C_K genes. However, other cell lines contained this sequence in rearranged contexts, suggesting that any deletion model of immunoglobulin V-J joining, as well as V gene mapping, must take into account the possibilities of stepwise rearrangements and reintegration of "deleted" DNA.

INTRODUCTION

Immunoglobulin genes require chromosomal DNA rearrangements for gene activation (1,2). In kappa light chain producing cells, for instance, one of several hundred variable (V) region gene segments has been brought into proximity of the single constant (C) region gene segment via one of five J (for joining) gene segments (1,2). One model for this V-J joining event proposes that the DNA regions separating V and J segments in the germline genome are excised from the chromosome during antibody gene rearrangement (the deletion model)(1,2). This hypothesis is supported by the absence of some germline variable region gene segments and some J region segments in certain antibody producing cells (3).

If the deletion model of V-J joining is correct, it should be possible to map the relative chromosomal positions of V_K gene families by determining, through Southern blot analysis, those V_K genes present and absent in various antibody producing cells (3). Such determinations are simplest to

interpret in cell lines that exhibit only a single, rearranged kappa chromosome since this circumvents the question of gene dosage in cells having multiple rearranged or germline chromosomes (4). We present here a V_K gene map obtained by analysis of certain selected myeloma and B lymphoid cell lines from inbred mice using Southern blot hybridization (5). In determining the map, we have assumed that the deletion model is correct and that DNA sequences between V and J genes are eliminated during the joining process. In contradiction with this model, however, we find that sequences 5' of rearranged J_K gene segments are retained in some cell lines. We discuss the implications of this finding regarding both the V_K gene map presented and the molecular mechanisms involved in V-J joining.

MATERIALS AND METHODS

The myelomas (MOPC167, MOPC21, NS-1n) and B-cell lymphomas (ABE8.1-2, CH1, CH2) used in this study were maintained as serially passaged tumors or as tissue culture lines. MOPC167, MOPC21, NS-1n and ABE8.1-2 are derived from Balb/c mice while CH1 and CH2 are derived from B10 mice.

The DNAs used as nick translated probes in Southern filter hybridization are listed and described in Table 1. Genomic DNA isolation and Southern blots were performed as described previously (4).

RESULTS AND DISCUSSION

The myeloma MOPC167 exhibits only a single rearranged kappa gene expressing V_{M167} (4,6). No germline kappa locus remains in this cell line. Consistent with the deletion model of antibody gene rearrangement, two V_K gene families, V_{M11} and V_{M21}, have been deleted from the MOPC167 genome (Table 1, Fig. 1A). This implies that the V_{M167} gene maps 5' of these families. The V_{M167} gene may, in fact, be toward the 5' end of the kappa locus since all myelomas, hybridomas, and B cell lines that we have tested retain the V_{M167} gene (4,7 and Table 1).

The myelomas, MOPC21 and NS-1n, also provide genomes amenable to analysis for V_K gene deletion. MOPC21 exhibits two rearranged C_K genes; one which is expressed and one which is a non-expressed "aberrant" gene (8,9,10). NS-1n, a variant cell line derived from MOPC21, represents only the aberrant gene in MOPC21, having lost the MOPC21 expressed allele (9). In NS-1n, the aberrant gene has apparently been duplicated; the two copies can be distinguished by an $\sim$1 kb deletion between J_4 and C_K (9). The rearranged aberrant genes in MOPC21 and NS-1n exhibit variable region segments identical

to the sequenced portion of the V_K polypeptide expressed in the myeloma ABPC-20 (V_{A20}), but are not functional due to a coding frameshift resulting from a misalignment during V-J joining (10).

Both MOPC21 and NS-1n myelomas retain the germline V_{M167} and V_{M11} gene families (Table 1). This suggests that these families are located 5' of the two V_K genes, designated as $V_{M21(x)}$ and $V_{A20(x)}$, that have undergone V-J joining in the myelomas. Analysis of MOPC21 and NS-1n DNA with V_{M21} and V_{A20} probes indicates that these families are closely spaced in the kappa locus. Six major hybridization bands represent the V_{A20} family in germline DNAs (Table 1 and Fig. 1E). Of these, NS-1n retains only $V_{A20(5)}$, in addition to two aberrant rearranged $V_{A20(x)}$ genes (Fig. 1E). MOPC21, on the other hand, retains all the V_{A20} bands except $V_{A20(3)}$ and exhibits a single aberrant $V_{A20(x)}$ gene (Fig. 1E).

We observe about six major V_{M21} hybridization bands in germline DNAs (Fig. 1D). All of these bands are also observed in MOPC21 and NS-1n DNAs; in addition MOPC21 exhibits the rearranged expressed $V_{M21(x)}$ gene (Fig. 1D). Apparently the germline counterpart of the $V_{M21(x)}$ rearranged gene comigrates with other germline V_{M21} genes since all germline V_{M21} hybridization bands are represented in MOPC21 DNA[11].

Once again, the data obtained with V_{A20} and V_{M21} are consistent with the deletion of variable region gene segments during V-J joining. The data also suggest that the unrelated V_{A20} and V_{M21} gene families are closely spaced and even interspersed in the kappa locus. The indicated map of this region is shown in Fig. 2E. $V_{A20(3)}$ is at the 3' end of the map since it is absent from both MOPC21 and NS-1n. $V_{M21(x)}$ is next since MOPC21 retains all V_{M21} and V_{A20} genes except $V_{A20(3)}$. Four V_{A20} genes are placed 5' of $V_{M21(x)}$ since they are present in MOPC21 but absent in NS-1n. The germline $V_{A20(x)}$ gene is the furthest 5' of these four genes. Upstream from $V_{A20(x)}$ we place $V_{A20(5)}$ and all the remaining V_{M21} genes since these are present in both MOPC21 and NS-1n. Their relative order cannot be determined.

The map of the V_{A20} and V_{M21} gene families that we obtain by deletion analysis is consistent with the general notions of V_K gene family clusters (12) although the indicated interspersion of unrelated families has not been detected previously. Additional data that we have obtained, however, may moderate this observation and are discussed below.

A final B-cell line, ABE-8.1-2, yields information concerning the V_K genetic map. ABE-8.1-2 exhibits a single rearranged C_K gene and no germline C_K genes, yet retains the V_{M167}, V_{M11} and V_{M21} gene families (Table 1).

Table 1: Summary of Kappa Gene Maps[a]

Cell type	Probe	Result
Kidney	C_κ[b]	1 germline
	deletion[c]	1 germline
	$J_{\kappa 3\text{-}4}$[d]	present
	V_{M167}[e]	1 germline
	V_{M11}[f]	3 germline
	V_{M21}[g]	6 germline
	V_{A20}[h]	6 germline
MOPC167 myeloma	C_κ	1 rearranged
	deletion	absent
	V_{M167}	1 rearranged
	V_{M11}	absent
	V_{M21}	absent
MOPC21 myeloma	C_κ	2 rearranged
	deletion	1 rearranged
	$J_{\kappa 3\text{-}4}$	present
	V_{M167}	1 germline
	V_{M11}	3 germline
	V_{M21}	1 rearranged 6 germline
	V_{A20}	1 rearranged no. 3 absent nos. 1,2,4,5,6 germline
NS-1n myeloma	C_κ	2 rearranged (same, except 1 kb deletion)
	deletion	absent
	$J_{\kappa 3\text{-}4}$	present
	V_{M167}	1 germline
	V_{M11}	3 germline
	V_{M21}	6 germline
	V_{A20}	2 rearranged (same, except 1 kb deletion) no. 5 germline, all others absent

Table 1 - continued

Cell type	Probe	Result
CH1 B lymphoma (λ producer)	C_K	2 rearranged
	deletion	absent
	V_{M167}	1 germline
CH2 B lymphoma (λ producer)	C_K	none
	deletion	2 rearranged
	J_{K3-4}	absent
	V_{M167}	1 germline
ABE-8.1-2 B lymphoma	C_K	1 rearranged
	deletion	2 rearranged[j]
	J_{K2-3}[i]	absent
	V_{M167}	1 germline
	V_{M11}	3 germline
	V_{M21}	6 germline

a Data obtained from Southern blot hybridization with the indicated probes. Minor hybridization bands (partial cross reaction) are not listed here.

b C_K cDNA cloned in pBR322, exclusive of J_K (9).

c Subcloned Hind III fragment 5' of J_1, derived from a genomic germline DNA clone (9), see Fig. 2A.

d AccI fragment comprising J_{K3} and J_{K4} excised from a genomic clone.

e Subcloned V_{M167} from a MOPC167 κ cDNA clone obtained from Early and Hood (17), exclusive of J_K, inclusive of leader peptide sequence (6).

f V_{M11} - C_K cDNA cloned in pMB9, obtained from R. Perry.

g V_{M21} cDNA exclusive of J_K cloned in pBR322 (9).

h. 300 base pairs excised from the 5' end of a genomic clone of the nonfunctional gene of MOPC-21 (9). It contains most of the leader sequence, including the leader intron (see Fig. 2D). The V region of this gene has been shown to be identical to the V_K region produced by the myeloma ABPC-20 (10).

i. AvaII fragment comprising J_{K2} and J_{K3} excised from a genomic clone.

j. While three bands can be detected on the BamHI Southern blot shown in Fig. 1B, the 8 kb band is relatively weak and only two bands are observed with EcoRI digestion (not shown).

In accordance with the deletion model, therefore, we have positioned V_{ABE8} at the most 3' location in our map closest to the C_K gene segment (Fig. 2F).

The Southern blot data described above allow construction of a tentative V_K family (see Fig. 2F). The finding of V_{M11} 5' of V_{A20} is consis-

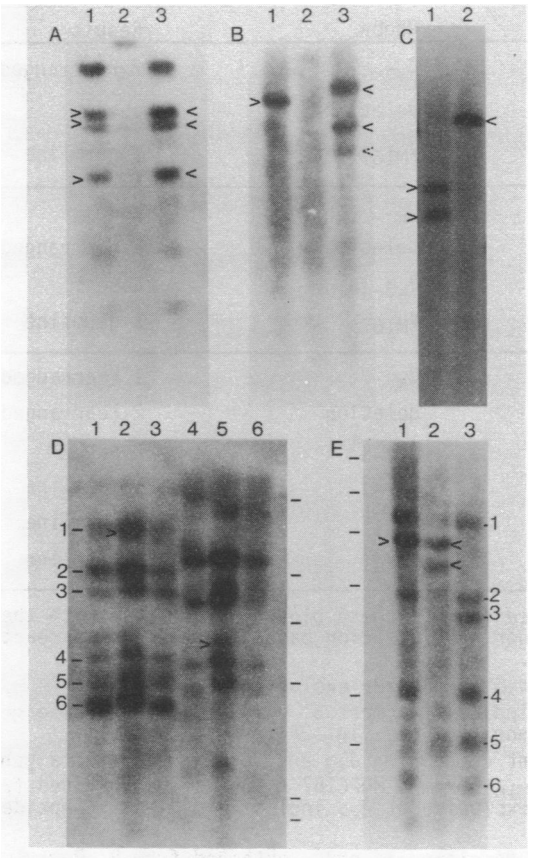

Figure 1. Southern blot analysis of kappa genes. A. 1 - Kidney, 2 - MOPC167 and 3 - ABE-8.1-2 DNAs digested with EcoRI and hybridized with V_{M11} - C_K probe. Kidney and ABE-8.1-2: 15.2 kb C_K band; 9, 8, and 6 kb V_{M11} bands (arrow heads). MOPC167: ~23 kb C_K band, no V_{M11} bands. B. Same DNAs as A., digested with BamHI, hybridized with "deletion" probe. Kidney: 13 kb band, MOPC-167: no band, ABE8: 15 kb and 10 kb bands (arrow heads); there may also be a 8 kb band (stippled arrow). C. CH2 (1) and kidney (2) DNAs, digested with EcoRI, hybridized with "deletion" probe. CH2: 7.4 and 5.8 kb bands; kidney: 15.2 kb band. D. Kidney (lanes 1 and 4), MOPC-21 (lanes 2 and 5), and NS-1n DNAs (lanes 3 and 6) digested with EcoRI (1-3) and BamHI (4-6) and hybridized with V_{M21}. The bars on the left are size markers (HindIII digested λ DNA), from top to bottom: 23.7, 9.5, 6.7, 4.3, 2.3, and 2.0 kb. Arrows point to the rearranged V_{M21} ($V_{M21(x)}$) genes in MOPC21 (15.6 and 5.2 kb respectively). The nos. next to the kidney DNA lane indicate the major V_{M21} genes. E. MOPC21 (1), NS-1n (2) and kidney (3) DNAs digested with both BamHI and EcoRI and hybridized with the V_{A20} probe. The nos. next to the kidney DNA lane indicate the major germline V_{A20} bands. Size markers are indicated as in D. Arrows indicate the rearranged V_{A20} ($V_{A20(x)}$) genes in MOPC-21 (6.9 kb) and NS-1n (6.9 and 5.9 kb).

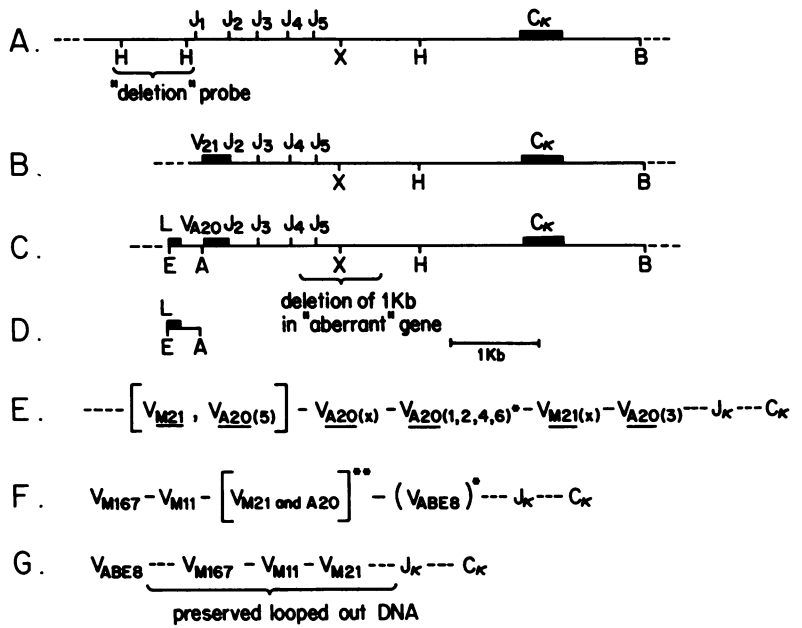

Fig. 2. Mapping of kappa genes. A-D. Restriction enzyme maps of germline and rearranged C_K genes (modified from (9)). L = leader peptide sequence. Restriction endonucleases are: E = EcoRI, A = AvaII, H = HindIII, X = XbaI, B = BamHI. A. Germline C_K gene. B. MOPC21 rearranged expressed V_{M21}-C_K gene. C. MOPC21 and NS-1n rearranged aberrant V_{A20}-C_K gene. One copy of the aberrant gene in NS-1n has a 1 kb deletion as indicated by a bracket. D. 5' fragment of clone shown in C (V_{A20} probe). E-G. Germline V_K maps. E. Map of the cluster of V_{M21} and V_{A20} genes. * The most 5' of these is the $V_{A20(x)}$ gene expressed in NS-1n and MOPC21. F. Map of all V_K genes analyzed in this study. *V_{ABE8} is located 3' of all V_K genes analyzed as indicated by deletion mapping. However, an alternative arrangement as indicated in G, may be possible (see text). **A fine map of V_{M21}-V_{A20} is shown in E. G. Possible alternative map location of the V_{ABE8} gene. A sequence 5' of J_1 is preserved in two rearranged copies in this cell line. This might be located together with the V_{M167}, V_{M11}, and V_{M21} genes in a preserved loop-out segment of DNA.

tent with data of Seidman et al. (3). They mapped the position of VM11 relative to V_{M321} which belongs to the same V_K family (12) as V_{A20}.

In addition, the data outlined above, obtained using V-region probes, is entirely consistent with the deletion model of immunoglobulin gene rearrangement. In order to further test this model, we analyzed six cell lines for the presence or absence of sequences just 5' of the J_K region (designated as the "deletion" probe in Table 1, see footnote c). In accordance with the deletion model, we did not find this sequence in the myeloma

MOPC167 (Fig. 1B), in the myeloma NS-1n or in the B-cell line CH1 (which has two rearranged C_K genes)(Table 1). However, unexpected results were obtained with other cell lines. MOPC21, as described above, has two rearranged C_K genes, yet retains sequences 5' of J_1 (Table 1). ABE-8.1-2 has only a single rearranged C_K gene yet retains two copies of sequences 5' of J_1 (Table 1, Fig. 1B). CH2, a lambda producing B-cell line, displays no copies of the C_K gene[13] yet also retains two copies of sequences 5' J_1 (Fig. 1C, Table 1). In each case, the retained genomic 'deletion' segments (our designation for genomic restriction fragments that unexpectedly retain sequences of 5' of J_1) are rearranged; that is, they do not comigrate with the sequences 5' of J_1 present in the germline genome (Table 1, Fig. 2B, C).

What is the state of sequences 5' of J_1 in cells which have not retained any germline C_K genes? Unexpected retention of such a sequence has also been observed by Steinmetz et al. (14). These authors cloned a genomic EcoRI fragment containing a sequence 5' of J_1 from the myeloma T (which exhibits two rearranged C_K genes). They showed that this sequence was contiguous to a flanking segment bearing striking similarity to sequences observed directly 3' of several V_K genes (14). Such a rearranged segment might be postulated to have been formed as a loop-out by-product from a V-J joining event (14).

The retained genomic 'deletion' segments that we observe in several cell lines exhibit characteristics that suggest they are related to the sequenced segment from the myeloma T. All exhibit restriction maps (data not shown) indicating that both HindIII sites located 5' of the J_K region (see Fig. 2A) are maintained. However, none exhibit the XbaI site just 3' of the J_K region (Fig. 2A); in addition, we have not found J_K sequences to be present on the genomic 'deletion' segments (Table 1). Thus, while some 'deletion' segments, such as those in the CH2 cell line, may have arisen by simple excision of C_K genes, this would involve a large region 5' of C_K, including the J_K locus. We believe that it is more likely that the generation of all genomic 'deletion' segments is linked to V-J joining; this is especially indicated for those found in cell lines maintaining C_K genes.

The absence of J_K sequences on the observed 'deletion' segments raises another interesting point. In ABE-8.1-2, for instance, restriction map analysis indicates that J_5 is the site of joining for the single rearranged kappa gene present (data not shown). Since no J_K sequences are found on either of the genomic 'deletion' segments in this cell line, there

is not a simple correlation between the deletion segments and the rearranged gene. This observation is similar to the situation in the myeloma T (14).

If the genomic 'deletion' segments containing sequences 5' of J_K are derived from looped out DNA in some connection with a V-J joining event, they could be present as a circular episome or be reintegrated into the genome. The latter appears more likely, since the 'deletion' segments seem to be present in every cell as indicated by their quantity on Southern blots (Fig. 1B, C). It will be interesting to determine whether these segments, which seem to possess some features of transposons (15), remain stable in these cell lines. An alternative possibility, that 'deletion' segments result from sister chromatid exchange during V-J joining, seems less likely because homologous DNA regions generally thought to be necessary for exchange are not found associated with V and J segments.

Taken together, our results and those of Steinmetz et al. (14) suggest that V-J joining may not be a one-step process. Instead, a family of V_K genes may first be brought into the vicinity of J_K, followed by a subsequent final V-J joining event - a situation similar to that postulated for the assembly of the D segment in heavy chain genes (16). Alternatively, V-J joining could involve the looping out of a single fragment of DNA, only a portion of which would have the potential for reintegration into the genome. Several other schemes can easily be envisaged.

The finding of retained genomic 'deletion' segments in certain cell lines casts some doubt on the reliability of V gene mapping. Is it not possible that somatic rearrangements <u>within</u> the V gene locus might obscure the germline arrangement of these genes? If deletion segments represent only the retention of limited genomic areas near the C_K locus, the maps we present are likely to be correct. In this regard it is noteworthy that the map positions for V_{M167}, V_{M11}, V_{M21}, and V_{A20} have been consistent in ten, six, four, and three cell lines, respectively (this paper and refs. 4,7,8). In each case, V_K gene families were deleted from haploid cells expressing upstream V_K genes and were present in those expressing downstream genes. If, however, larger retained loop-out segments are proposed, then the order of some V_K gene families may be altered (Fig. 2G). The indicated interspersion of V_{M21} and V_{A20} families could also be invalidated; for instance, it is possible that the gene order is $V_{21(1-6)}$ ... $V_{A20(1-6)}$ ... J_K if, in the rearrangement of $V_{M21(x)}$, the V_{A20} genes are looped out and reinserted elsewhere in the genome except for $V_{A20(3)}$ which is lost (see Table 1). Other possibilities may also exist. If even further genetic instability of

the immunoglobulin gene locus is postulated, with deletions and reinsertion of sequences occurring in various areas and fashions, deletion mapping of V genes becomes very tentative and, in addition, the evidence that might have previously supported the deletion model for antibody gene rearrangement over other possible mechanisms (1,2,3) must come into question. Further evaluation of the deletion segments found in some cell lines may resolve this point.

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