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Comparison of mRNA precursors in plasmacytomas producing closely related κ chains

(gene rearrangement/J segments/transcription unit/processing)

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ABSTRACT **To help elucidate the mechanisms of formation and expression of active immunoglobulin genes, we have made a systematic study of the processing patterns of the mRNAs that code for a closely related family of κ chains. Among the members of this family, represented by the $V_κ21$ group of plasmacytomas, are examples in which six different germ-line *V* genes are joined to four different J segments in various combinations. The mRNA precursors were identified by hybridizing a cloned κ-cDNA probe to poly(A)-containing nuclear RNAs that were size fractionated on methylmercury-agarose gels and transferred to diazotized paper. Based on the length of the segment excised in the last detectable processing step, which presumably represents the removal of the J–C intron, the precursor patterns were classified into four primary categories that correlated well with the type of J segment being expressed. The J segments were thus located at distances ranging from 2.4 to 4.8 kilobases from the constant-region gene. Different *V* genes joined to the same J segment exhibited similar precursor patterns, suggesting that the size of the translocated V segment may be relatively uniform among members of the $V_κ21$ group. A large (9.1 kilobases) component, which is likely to represent a primary transcription product, was observed in all processing categories, regardless of which J segment was being utilized. This surprising observation leads to some interesting predictions about the mechanism of V–J translocation.**

During the differentiation of B lymphocytes, the DNA elements specifying the variable (V) and constant (C) portions of immunoglobulins are rearranged from their germ-line configuration to form transcriptionally active units that generate precursors of specific immunoglobulin mRNAs (1–5). For each species of κ light chain, a specific $V_κ$ gene is transposed to one of several $J_κ$ segments located 2–5 kilobases (kb) upstream from the $C_κ$ gene. The multiplicity of distinctive $V_κ$ genes, their combinatorial joining with the multiple J segments, and the occurrence of somatic mutations can account for the great diversity of κ chains that is observed in the mouse (6–9). The intervening sequence between the $C_κ$ segment and the expressed J segment (C–J intron) is apparently transcribed and then excised as part of the subsequent processing of the immunoglobulin mRNA precursors (10, 11).

Although these events can be described in broad outline, the mechanism of translocation and the nature of the translocated units are unknown. Moreover, the source of the DNA elements that serve as the initiation sites for the active transcription units is also obscure. One effective way of attacking this problem is to examine the various DNA segments before and after translocation (1–3, 6, 7). A complementary approach is to study the processing pattern of the immunoglobulin mRNA precursors. Because processing patterns are related to the excision of introns and splicing together of the various structural gene elements (12), they reflect the underlying organization of the *expressed* genes. Inasmuch as transcription of immunoglobulin gene sequences in myeloma cells appears to be largely directed toward the production of functional mRNAs (4, 5, 11, 13), this approach should avoid problems with nonproductive DNA rearrangements (2, 3, 7).

Therefore, we have analyzed the mRNA precursors in a series of plasmacytomas producing closely related κ chains. Members of this family, called the $V_κ21$ group, all produce κ chains containing a similar sequence for the first 23 residues (FR1) (9). On the basis of distinctive and recurring differences beyond FR1 (residues 24–95), this group has been divided into six major subgroups designated $V_κ21A$ through -F, each of which is believed to be derived from a separate germ-line *V* gene (8). The individual members within a subgroup may be associated with any one of several different J segments, as evidenced by the amino acid sequence of residues 96–107 (9). Thus, we can compare the mRNA precursor patterns among plasmacytomas that are presumably expressing different germ-line *V* genes with the same J segment, and the same *V* gene with different J segments. We have observed four distinctive precursor patterns which are generally correlated with particular types of J sequence but *not* with the type of *V* gene being expressed. All four patterns exhibit a common 9.1-kb component, presumed to be the initial product of a uniform $V_κ21$ transcriptional unit. These results have enabled us to order the positions of the J sequences and to suggest some features that should be incorporated into models for the formation of active κ-chain transcriptional units.

MATERIALS AND METHODS

The NZB plasmacytomas, provided by Martin Weigert of this institute, are propagated as solid tumors in (NZB × BALB/c)F, hybrid mice. Nuclei were prepared from freshly excised tumors by the citric acid procedure, and RNA was extracted from them by the hot-phenol method (14, 15). Poly(A)-containing RNA was purified by oligo(dT)-cellulose chromatography, size fractionated by electrophoresis on methylmercury-agarose gels, and transferred to diazobenzyloxymethyl-cellulose paper (5, 16, 17). The components containing nucleotide sequences coding for the κ and $γ_{2b}$ C regions were identified by hybridization to ^{32}P-labeled nick-translated recombinant plasmids pκ(11)[24] and p$γ_{2b}$(11)[7], respectively (5). The $γ_{2b}$ probe has sufficient homology with $γ_{2a}$ sequences (18) to enable the identification of $γ_{2a}$ mRNA precursors as well as $γ_{2b}$ mRNA precursors. The sizes of the various components (in kilobases) were determined from strictly linear plots of the logarithm of molecular weight against mobility, using ethidium bromide-stained pre-rRNAs and rRNAs as standards (5, 16).

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Abbreviations: V, variable; C, constant; kb, kilobase(s).

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RESULTS

The panel of $V_\kappa 21$ plasmacytomas used in this study included members of all six major subgroups (A through F) in association with five basic types of J segment, as defined by the amino acid sequence of all or most of the V region—i.e., residues 1–107 (Table 1). In addition, we included several examples that produce κ chains related to $V_\kappa 21$ as judged by reactivity with group-specific antisera and partial amino acid sequence data but have not yet been assigned to a subgroup of J type. Two plasmacytomas producing non-$V_\kappa 21$ κ chains were also examined.

The κ mRNA precursor patterns characteristic of the various plasmacytoma lines are shown in Figs. 1 and 2. All of the nuclear RNA samples exhibited an intense 1.2-kb band corresponding to the mature κ chain mRNA; these bands were of identical size in all samples (see autoradiographs made with relatively brief exposures, Fig. 1 *right center*). Various larger components were observed, those most frequently occurring being approximately 9.1, 6.0, 5.5, 5.0, 4.5, and 3.6 kb long. The autoradiographic exposure times were varied in order to compensate for variations in the relative amounts of these components among the different tumors, thus enabling us to maximize the number of detectable precursors. That the observed difference in the precursor patterns is not due to variable degradation of some of the nuclear RNA samples was shown by melting off the κ probe and reannealing the same RNA samples with a probe capable of recognizing the γ_{2a} or γ_{2b} class heavy chain mRNA precursors (Fig. 1 *bottom*). Several of the nuclear RNA samples contained heavy chain mRNA precursors that were substantially larger than their light chain counterparts. For example, in PC-7183 and PC-1229, in which the largest observable κ mRNA precursors are 4.5 and 6.0 kb, respectively, there were heavy chain mRNA precursors 8.8 and 12 kb long. These heavy chain mRNA precursors were as discrete and in the same size range as those found in tumors in which large (9.1 kb) κ mRNA precursors were present (e.g., PC-9245, PC-7132, PC-7175, and PC-6308). Thus, it is clear that the variation in precursor patterns is not due to variable degradation of nuclear RNA preparations.

Some tumors (e.g., PC-7210 and PC-2960) produced a 0.8-kb component in addition to the 1.2-kb mRNA (Fig. 3). This component is similar in size to the C region fragment mRNA that is produced by MPC-11 myeloma cells (19). As we demonstrated for MPC-11 cells (5), the precursor patterns are apt to be more complicated in such tumors. Thus, the 4.8-kb component in PC-7210 and the 1.9-kb component in PC-2960 may be related to the production of a 0.8-kb fragment mRNA.

Our ability to distinguish the size differences between the various precursor components is clearly seen in Fig. 2 in the adjacent tracks containing RNA from PC-3741 (5.5 and 5.0 kb), PC-6684 (6.0 kb), PC-7183 (4.5 and 3.6 kb), and PC-7043 (9.1, 5.1, and 4.1 kb). This discrimination was further verified by observing the band broadening (or lack thereof) with equimolar mixtures of an RNA with a 6.0-kb band (PC-2880) and RNA from four other tumors exhibiting bands at 6.0 kb (PC-6308), 5.5 kb (PC-7132 and PC-3741), and 4.5 kb (PC-7183) (Fig. 4).

Table 1. mRNA processing patterns in $V_\kappa 21$ plasmacytomas

J type	V subgroup	Tumor	Precursor pattern	Final excision, kb	Category
1	A	PC-2880	6.0 → 1.2	4.8	I
1	A	1229	6.0 → 1.2	4.8	I
1	D	6308	9.1 → 6.0 → 1.2	4.8	I*
1	D	7769	6.0 → 1.2	4.8	I
1	D	7210	6.0 / 4.8 / 1.2/0.8		I
$1s^1$	E	6684	6.0 → 1.2	4.8	I
$1s^2$	E	7940	9.1 → 6.0 → 1.2	4.8	I*
2	B	9245	9.1 → 5.5 → 5.0 → 1.2	3.8	II*
2	C	3741	9.1 → 5.5 → 5.0 → 1.2	3.8	II*
2s	A	7132	9.1 → 5.5 → 5.0 → 1.2	3.8	II*
3	D	7043	9.1 → 5.1 → 4.1 → 1.2	2.9	III*
4	B	4050	9.1 → 6.0 → → 1.2	4.8	I*
4	D	7183	4.5 → 3.6 → 1.2	2.4	IV
4	E	7175	9.1 → 4.5 → 3.6 → 1.2	2.4	IV*
4	F	2485	4.5 → 3.6 → 1.2	2.4	IV
4	?	2154	4.5 → 3.6 → 1.2	2.4	IV
x	?	2413	9.1 → 6.0 → 5.2 → 1.2	4.0	?
?	?	7461	9.1 → 6.0 → 1.2	4.8	I*
?	?	4999	9.1 → 6.0 → 1.2	4.8	I*
?	?	2960	5.3 / 1.9 / 1.2/0.8		?
Non-$V_\kappa 21$		3852	9.4 → 5.0 → 1.2		II
Non-$V_\kappa 21$		8916	13 → 8.4 → 7.4 → 4.1 → 1.2		III

The V subgroups and J types were determined according to the amino acid sequence of residues 1–95 and 96–107, respectively (9). The amino acids in positions 96, 100, and 106 were, respectively, Trp, Gly, Ile for type J_1; Tyr, Gly, Ile for J_2; Phe, Ser, Ile for J_3; and Leu, Ala, Leu for J_4. J_x resembles J_1 at these three positions, but has Asp instead of Lys at position 103 and Glu instead of Lys at position 107. The J types labeled $1s^1$, $1s^2$, and 2s could arise by variations in the site of somatic joining of the V and J segments. $1s^1$ and $1s^2$, respectively, had Arg and Pro instead of Trp at position 96; 2s had an additional Pro inserted between the Pro at position 95 and the Tyr normally found at position 96. The Js were identical at the other positions. The precursor patterns are based on reproducible and clearly discernible bands (see Figs. 1–4). The final excision represents the difference between mature (1.2-kb) mRNA and the next larger component. The processing patterns are categorized primarily (Roman numeral) according to the size of the final excision and secondarily (asterisk) according to whether a 9.1-kb component was observed.

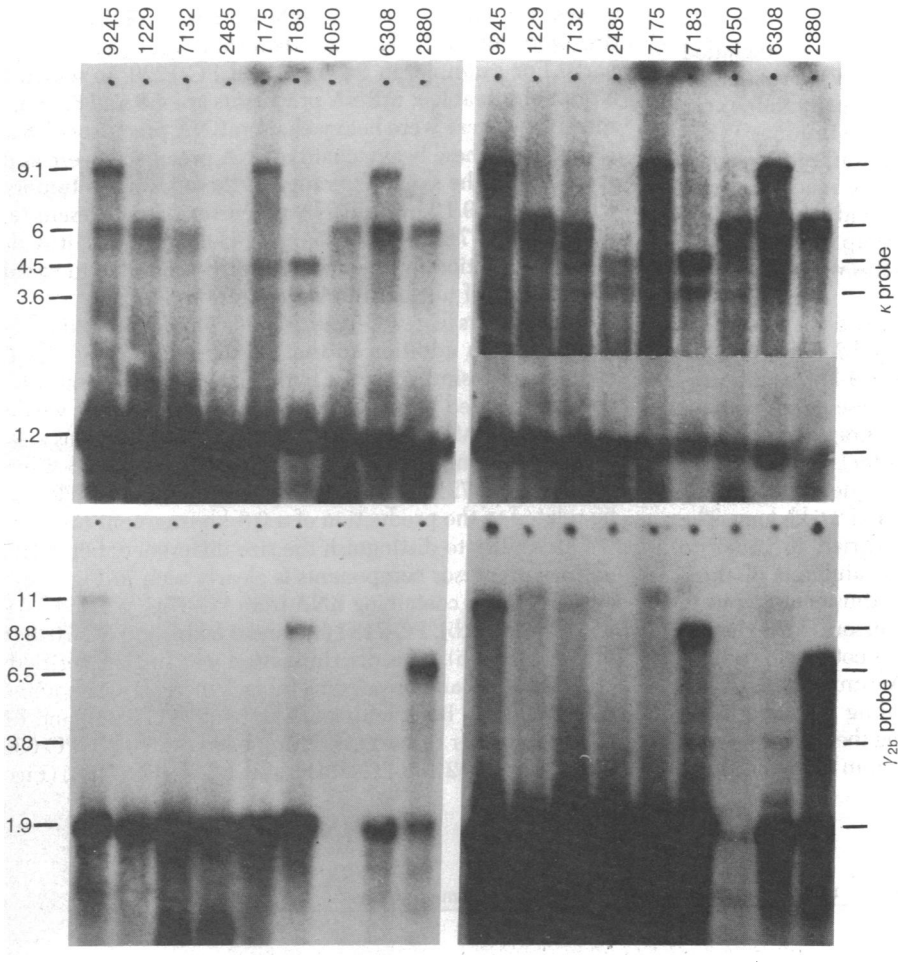

FIG. 1. Analysis of immunoglobulin mRNA precursors in a series of nine plasmacytomas. Poly(A)-containing nuclear RNAs (10 μg each) were fractionated by electrophoresis on methylmercury-agarose gels, transferred to diazobenzyloxymethyl paper, and hybridized with a ^{32}P-labeled cloned probe (≈300 cpm/pg) containing κ(V+C) sequences (*Upper*). After exposure of the autoradiographs, the κ probe was melted off by a 30-min incubation in 99% formamide at 68°C, and the paper was rehybridized with a ^{32}P-labeled cloned probe containing γ_{2b} (C) sequences (*Lower*). Autoradiographic exposure was for 16 hr (*Left*), 40 hr (*Right Upper* and *Lower*), or 7 hr (*Right Center*). Size, in kb, is shown at left.

All of the samples were run at least twice, and in some cases (e.g., PC-2880, PC-3741, PC-4050, PC-6308, and PC-6684) several RNA samples extracted from different batches of the same tumor line were compared. The overall variance of the size measurements of any particular component was about ±0.1 kb. Essentially no qualitative differences in pattern were observed among the various tumor batches. For example, the 9.1-kb component was clearly evident in each of four different batches of PC-6308 but never was observed in any of four batches of PC-2880.

Comparison of the mRNA precursor patterns in 22 different plasmacytoma lines revealed some interesting regularities when the data were grouped according to J type (Table 1). From the results of analyses of κ mRNA processing in MOPC-21 cells (10, 11) it might be inferred that the final excision—i.e., the production of the mature (1.2 kb) mRNA from the next larger precursor component—would correspond to the removal of the J–C intron. Accordingly, we categorized the precursor patterns

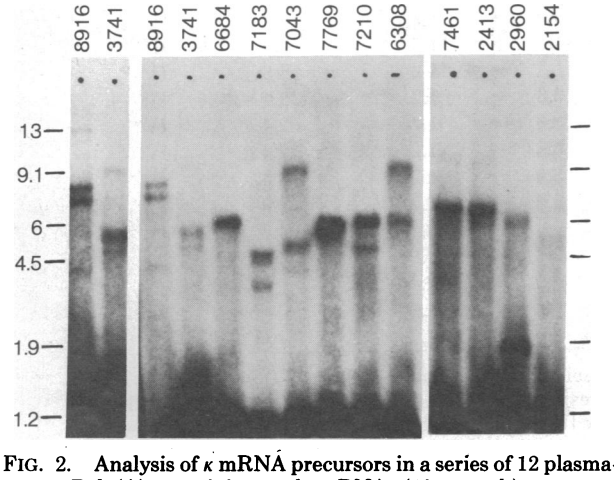

FIG. 2. Analysis of κ mRNA precursors in a series of 12 plasmacytomas. Poly(A)-containing nuclear RNAs (10 μg each) were analyzed as in Fig. 1. Autoradiographic exposure was 40 hr (*Left*) or 16 hr (*Center* and *Right*). The mobility in the gel of the *Right* panel was slightly retarded relative to that in *Center* and *Left*. Size, in kb, is shown at left.

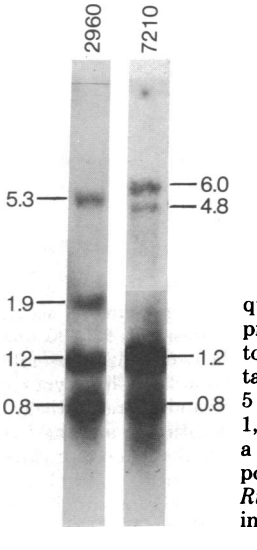

FIG. 3. Analysis of κ mRNA sequences in plasmacytoma lines that produce a 0.8-kb fragment in addition to the 1.2-kb κ mRNA. Poly(A)-containing nuclear RNAs (10 μg of 2960; 5 μg of 7210) were analyzed as in Fig. 1, except that electrophoresis was for a shorter time. Autoradiographic exposure was 14 hr (*Left*), 37 hr (*Upper Right*), or 14 hr (*Lower Right*). Sizes, in kb, are shown at sides.

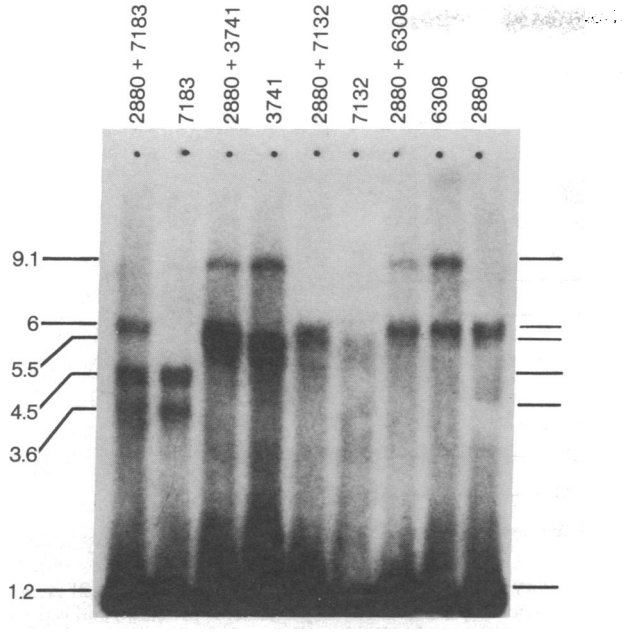

FIG. 4. Verification of resolution in analysis of κ mRNA precursors. Poly(A)-containing nuclear RNAs (10 μg each) of five plasmacytomas or mixtures of 5 μg of one plasmacytoma (PC-2880) and 5 μg of one of the other four were analyzed as in Fig. 1. Autoradiographic exposure was 40 hr.

primarily according to the size of this excision (Roman numeral) and secondarily according to whether a 9.1-kb component was observed (asterisk). For the most part there is a good correspondence between the size of final excision and the J type being expressed: 4.8 kb for J_1, 3.8 kb for J_2, 2.9 kb for J_3, and 2.4 kb for J_4. Given the presumptive relationship between the sizes of these excisions and the lengths of the J–C introns, we would predict that the gene segments encoding these four J types are located at corresponding distances from the C_κ gene. One exception to this correlation was noted. Tumor PC-4050 exhibited a category I* processing pattern in spite of the fact that its expressed J sequence is J_4. PC-2413, which has an expressed J segment (J_x) that is unique among all the $V_\kappa 21$ examples so far examined, may also have a distinctive processing mode.

The κ chains produced by plasmacytomas 6684, 7940, and 7132 have J_1 or J_2 sequences except for unique substitutions at position 96, which comprises part of the third complementarity-determining region (9). Because these substitutions may be attributable to variations in the site of joining of the germ-line V and J segments (20), we have tabulated the J types as somatic variants of J_1 and J_2 rather than as additional germ-line J sequences. The fact that the mRNA precursor patterns in these tumors are in the same categories as those producing J_1 or J_2 proteins (rather than representing additional categories) tends to support this notion.

There was no apparent relationship between the mRNA precursor pattern and the particular $V_\kappa 21$ gene being expressed. This lack of correlation extends even to the presence or absence of the 9.1-kb component. Thus, members of the A subgroup are found in categories I and II*, members of the D subgroup in categories I, I*, III*, and IV, and members of the E subgroup in categories I, I*, and IV*. These observations indicate that the structural organization of the κ chain transcriptional unit can be similar among $V_\kappa 21$ members, even when different $V_\kappa 21$ genes are translocated. More extensive variability might be anticipated when comparing V_κ genes belonging to more distantly related families, as is evidenced by the substantially different patterns found for PC-8916 and PC-3852.

DISCUSSION

The order of J segments inferred from our analysis of mRNA precursor patterns in a series of NZB mouse plasmacytomas coincides with that recently found by direct sequencing of the J region portion of germ-line DNA from the BALB/c mouse (20, 21). The DNA sequencing studies indicate that the four J segments encoding the peptides that we have designated J_1 through J_4 are located approximately 3.9, 3.5, 2,9, and 2.6 kb from the C_κ gene. There is an additional J-like segment about 3.1 kb from the C_κ gene, but no myeloma expressing this J segment has as yet been found. The J–C intronic distances indicated by the mRNA precursor studies are similar for J_3 (2.9 kb) and J_4 (2.4 kb). However, the distances for J_1 (4.8 kb) and J_2 (3.8 kb) are significantly greater. This discrepancy may be attributable to a difference between BALB/c and NZB mice—e.g., different spacer lengths in the region between J_1 and J_3. This region could conceivably contain an additional J segment—e.g., J_x—in the NZB mouse. The concordance between the genetic structure of the germ-line J_κ region and the organizations of numerous expressed κ genes, as inferred from their mRNA precursor patterns, tends to validate our interpretation of these patterns, in particular our supposition that the final excision represents the removal of the J–C intron. This agreement also provides compelling evidence for the generality of the V–J translocation model (1, 2) in the formation of transcriptionally active κ genes. There is no obvious explanation for the tumor that produces a J_4 type peptide but exhibits a category I* mRNA precursor pattern. Whether this observation signifies the possibility of additional rearrangements within the J–C region or the expression of another C_κ gene (8) remains to be established.

One of the most striking features to emerge from our analysis is the occurrence of the 9.1-kb component in each of the processing categories. These components, the largest observed in $V_\kappa 21$ tumors, are likely to be primary transcription products of the active κ genes. They are not detected in about 40% of the plasmacytoma lines, either because there is a fundamental difference in the organization of the transcriptional units in these cases or because there are variations in the rapidity of early processing steps among the different tumor types. Since the blotting technique used in these studies can detect even a few mRNA sequences per cell (5), processing would presumably have to occur on growing transcripts, or at least prior to polyadenylylation, in order to preclude the observation of full-length precursor molecules. Previous studies of the processing of κ mRNAs provide one example, MOPC21, in which a large (≈10 kb) precursor is evident (4, 11) and another, MPC-11, in which the largest precursor observed is 5.3 kb (5). No larger pre-κ mRNA components were detected in MPC-11 cells, even when rapidly labeled nuclear RNA was examined (5). Thus, it is conceivable that the presence or absence of the 9.1-kb component among the $V_\kappa 21$ tumors reflects differences in gene organization. However, this question will not be clearly resolved until we definitively locate the sites of transcriptional initiation.

The results of this study suggest some features that should be incorporated into models for the formation of active κ-chain transcriptional units. In the context of the V–J joining model, our data indicate that the translocated V region-containing segment of DNA is of relatively uniform size for a closely related family of *V* genes, such as the $V_\kappa 21$ group. Thus, we observe essentially identical processing patterns for $V_\kappa 21A$, $V_\kappa 21D$, and $V_\kappa 21E$ genes joined to the J_1 segment; for $V_\kappa 21A$ and $V_\kappa 21C$ genes joined to the J_2 segment; or for $V_\kappa 21D$ and $V_\kappa 21F$ genes joined to the J_4 segment. The idea that each family of related V_κ genes has a relatively uniform translocatable element is consistent with the observed uniformity of their flanking sequences (6).

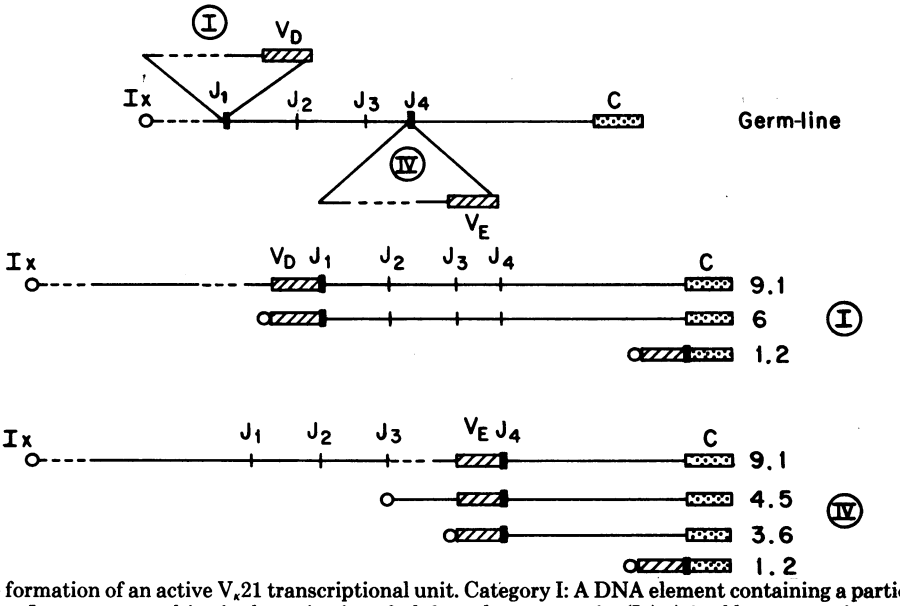

FIG. 5. Model for the formation of an active $V_\kappa 21$ transcriptional unit. Category I: A DNA element containing a particular $V_\kappa 21$ gene—e.g., $V_\kappa 21D$—is inserted next to a J_1 segment, resulting in the activation of a leftward promotor site (Ix). A 9.1-kb precursor is produced and is processed into a 6.0-kb intermediate and then into the mature RNA by excision of the J_1–C intron and suitable splicing. Category IV: A DNA element containing a particular $V_\kappa 21$ gene—e.g., $V_\kappa 21E$—is inserted next to a J_4 segment, resulting in the activation of Ix. A 9.1-kb precursor is produced and is processed via 4.5-kb and 3.6-kb intermediates into the mature mRNA.

Our finding of a common 9.1-kb precursor regardless of which J segment is expressed suggests that there may be a transcriptional initiation (promotor) site located at a fixed distance upstream from the J region, which is, in some way, activated by the translocation process. One model that would accommodate these facts involves the insertion of the *V* gene-containing elements into the J region (Fig. 5) in a fashion analogous to the invasion of a plasmid by a transposon (22). This model predicts that there should be J_1 sequences in precursors of J_4 mRNAs as well as J_4 sequences in precursors of J_1 mRNAs. Alternative models involving reciprocal recombination or deletion of J segments require a more complicated set of assumptions in order to account for the constancy in the size of the transcription unit.

At the RNA level, each of the J segments presumably has a similar feature which provides specificity for the excision–splicing event that brings the J and C encoding regions together (12). When unexpressed J sequences are transcribed as part of the mRNA precursors, it is conceivable that they might be recognized by the appropriate processing enzymes. However, this does not appear to happen; we do not observe any components that indicate splicing of the C region to an intermediate J segment. When additional precursors are observed—e.g., the 5.5-kb, 5.1-kb, and 4.5-kb components in category II, III, and IV patterns, respectively—they are larger than the predicted intermediate containing the J–C intron. These components probably signify a splicing event involving 5′ leader elements (11, 12). The removal of small introns, such as the 90–130 nucleotide element interrupting the sequence coding for the signal peptide (2, 20) would not be detectable in our analysis. Most likely, immunoglobulin mRNA processing proceeds in a 5′ → 3′ direction as is the case for rRNA (23) and ovalbumin mRNA (24).

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