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Transcription of mouse κ chain genes: Implications for allelic exclusion

(nuclear RNA/mRNA precursors/gene rearrangements/plasmacytoma cells)

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ABSTRACT **The nuclear RNA from a large variety of κ-producing plasmacytomas was size fractionated and analyzed with a series of cloned probes representing sequences encoding variable (V), joining (J), and constant (C) regions and selected intervening sequences. All of the plasmacytomas produce a nuclear RNA component that contains V_κ and C_κ sequences as well as the intervening sequence between J_κ and C_κ, and that has a distinctive size depending on which of the four J_κ segments is expressed (i.e., is present in the secreted κ chain). These RNAs are the precursors of κ mRNAs, which are transcribed from productively rearranged C_κ genes. Half of the plasmacytomas examined produce, in addition to a κ mRNA precursor, a discrete component of about 8.4 kilobases that contains C_κ and upstream flanking sequences but lacks the expressed V region sequence. The ability to produce this component is always associated with the persistence in the tumor genome of an unrearranged (germline) J_κ–C_κ region. In tumors rearranged at both κ loci the nonproductive allele is either transcriptionally silent or, in a minority of cases, transcribed and processed into a "fragment" mRNA lacking V region sequences. These results reveal that allelic exclusion can be effected at several levels of gene expression. They also provide some insight into the relative contributions of the V and C gene elements to this expression.**

The formation of an active immunoglobulin gene requires a reorganization of the germline genome to bring the elements coding for the variable (V) and constant (C) regions within a single transcriptional unit (1). In the case of mouse κ chains this reorganization appears to involve a deletion of the DNA that separates the expressed V_κ gene and any one of four different J_κ (joining) gene segments that are located from 2.7 (J_4) to 3.9 kilobases (kb) (J_1) upstream from the C_κ gene[§] (2, 3). Transcription of such a rearranged κ locus results in the formation of a pre-mRNA that contains the intervening sequence between the C_κ gene and the expressed J_κ segment (J–C intron) (4–7). This intron is subsequently excised during the processing of the κ mRNA. Because the length of the J–C intron is different for each of the four J_κ segments, plasmacytomas expressing different J_κ segments contain pre-mRNAs of characteristic and distinctive sizes (7). The observation that productive rearrangements are found on only one of the two chromosomes bearing the κ genes is thought to provide a molecular basis for the phenomenon of allelic exclusion (1, 8). However, there is presently some uncertainty about the nature and extent of rearrangements on the chromosome bearing the "nonexpressed" C_κ allele (8–11). Moreover, the relationship between the structural organization of the κ locus and its transcriptional activity is also obscure.

In several plasmacytomas a large (≈9 kb) C_κ-containing transcript is produced, which, in contrast to the pre-mRNAs described above, has a uniform size irrespective of which J_κ segment is expressed (7). We initially thought that this component was also an mRNA precursor and that its size uniformity might indicate an insertion-type mechanism for V-J joining. However, we now report further studies which demonstrate that this large component is, in fact, a transcript of the unrearranged (germline) C_κ allele. Out of the 30 different plasmacytomas examined, 15 produce this large transcript and demonstrably contain an unrearranged J_κ–C_κ region. These results have led to some general conclusions about the nature of allelic exclusion.

MATERIALS AND METHODS

Poly(A)$^+$ and poly(A)$^-$ nuclear RNA was isolated from plasmacytomas, separated on CH_3HgOH gels, and covalently bound to diazotized paper as described (6, 7). Four probes were used to identify immunoglobulin-related sequences. The C_κ and V_κ21 probes were derived from *Bam*HI/*Hin*dIII digests of the recombinant plasmid 5D10 (10), which contains the mRNA sequence of MOPC321. Fragments containing ≈0.3 kb of V region sequence and ≈0.6 kb of C_κ and 3′ untranslated sequence (Fig. 1*a*) were inserted into plasmid pBR322 and subcloned. *Hin*dIII/*Xba* I digestion of the *Eco*RI embryonic DNA fragment contained in the recombinant phage λCh4A-EC-5 (8) yielded a fragment containing about 1.1 kb of intervening sequence between J_4 and C_κ (IVS probe) and a fragment spanning the entire J region (Fig. 1*b*). A probe representing the J_2 segment and about 0.8 kb of flanking sequence was obtained from the latter fragment by *Hin*fI digestion. This fragment was ligated to *Bam*HI linkers, inserted into pBR322, and subcloned. Agarose gel electrophoresis was used to isolate the various restriction fragments. The probes were nick-translated with [α-^{32}P]dCTP to a specific activity of 200–400 cpm/pg and annealed with the paper-bound nuclear RNA. The RNA-papers were washed as described (7), then given a final stringent wash for 1 hr at 44°C in 50% formamide/60 mM NaCl/6 mM sodium citrate and prepared for autoradiography.

The sizes of RNA components containing immunoglobulin-related sequences were estimated relative to samples of plasmacytoma poly(A)$^-$ nuclear RNA. Such RNA contains rRNA and pre-rRNA components, which are readily visualized when stained with ethidium bromide (12). In our previous studies (6, 7, 12) we used values determined by electron microscopy as molecular weight standards for these rRNAs. Although these standards are sufficiently accurate for determi-

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

[§] The term "expressed" refers to the sequence that is present in the secreted κ chain. Only expressed J segments are enumerated. J_1, J_2, J_3, and J_4, respectively, correspond to J_1, J_2, J_4, and J_5 in ref. 3 and J_5, J_4, J_2, and J_1 in ref. 2.

nation of relative sizes, when we attempted to compare the absolute sizes with the sizes predicted from nucleotide sequencing of genomic DNA (3), it appeared that our values might be slight overestimates. Indeed, an overestimation of about 10% was indicated when we ran our standards together with *E. coli* 16S rRNA and MS2 phage RNA, the sizes of which are exactly known from sequence data, and with a set of denatured restriction endonuclease fragments of λ phage. Therefore, in the present work we have used standard values (1.8, 4.7, and 5.8 kb for the 18S, 28S, and 32S components, respectively) which are 10% lower than those used previously.

Plasmacytoma DNA was isolated (13), digested with *Hin*dIII (1 unit/μg for 4 hr at 37°C), separated on 0.6% agarose gels, and blotted onto nitrocellulose paper (Schleicher & Scheull) as described by Southern (14). After hybridization with the IVS probe the blots were washed as described by Jeffreys and Flavell (15).

RESULTS

In previous experiments (7) we identified the C_κ-containing nuclear RNA components of various $V_\kappa 21$ plasmacytomas by using the cloned probe $p\kappa(11)^{24}$ (6), which contains sequences encoding both V and C regions of the κ chain produced by MPC-11 cells, a member of the $V_\kappa 19$ group. Because the sequences encoding $V_\kappa 19$ and $V_\kappa 21$ groups do not cross-hybridize (16), the $p\kappa(11)^{24}$ probe exclusively recognizes the C_κ (and 3′ untranslated) sequences when used with $V_\kappa 21$ RNAs. For a more complete characterization of the sequence content of the nuclear RNA components we have carried out a series of blotting experiments with four additional probes (Fig. 1): (*i*) a probe for the V region expressed by MOPC321 cells, which recognizes the V regions of all members of the $V_\kappa 21$ group (16); (*ii*) a probe for the C_κ region (J_3 + C + 3′ untranslated segment of MOPC321), which, for these experiments, is essentially equivalent to the previously used $p\kappa(11)^{24}$ probe; (*iii*) a probe for 1.1 kb of the intervening sequence located between J_4 and C_κ, a sequence that is common to all expressed κ genes and absent from all expressed κ mRNAs; and (*iv*) a probe containing the J_2 segment and ≈0.8 kb of its flanking sequence, which, according to the deletion model (2, 3) should hybridize with κ mRNA precursors of J_1 and J_2 expressors, but not with the κ mRNA precursors of J_3 and J_4 expressors.

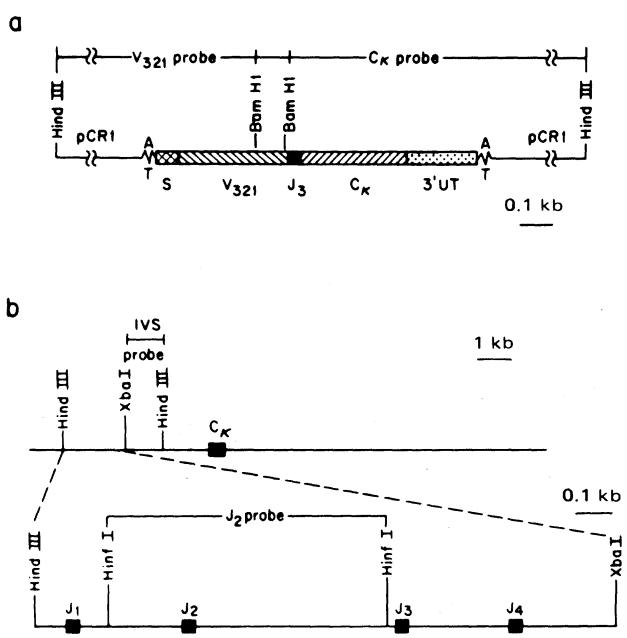


FIG. 1. Description of Ig sequence probes. (*a*) V_{321} and C_κ probes are cloned restriction endonuclease fragments derived from a pCR1 recombinant carrying MOPC321 κ mRNA sequences. (*b*) IVS and J_2 probes are purified restriction endonuclease fragments derived from a Charon 4A recombinant phage carrying the *Eco*RI fragment of BALB/c mouse embryo DNA that contains the C_κ gene. S, sequence encoding signal peptide; 3′ UT, 3′ untranslated sequence.

The results of such blotting experiments for a large variety of plasmacytomas are shown in Figs. 2 and 3 and interpreted in Fig. 4. The large 8- to 9-kb transcript (current estimate ≈8.4 kb) contains C_κ, IVS, and J_2 sequences, but not $V_\kappa 21$ sequences. Thus, contrary to our previous assumption, this component does *not* represent a transcript of the productively rearranged C_κ gene. Rather, the fact that it is of uniform size in tumors expressing any of the four J_κ genes, as well as its sequence composition, suggests that it is a transcript of the unrearranged (germline) C_κ allele. This contention is corroborated by DNA analysis (*vide infra*). The 5.3-kb, 5.0-kb, 4.4-kb, and 4.1-kb components, which are uniquely found in J_1, J_2, J_3, and J_4 expressors, respectively, have the properties expected of transcripts of the productively rearranged C_κ allele. These components all react with C_κ, $V_\kappa 21$, and IVS probes. As one would predict from the V–J joining model, the 5.3-kb (J_1) and 5.0-kb (J_2) components react with the J_2 probe, but the 4.4-kb (J_3) and 4.1-kb (J_4) components do not. The additional components of ≈4.3, 3.7, and 3.4 kb, which are uniquely observed in J_2, J_3, and J_4 expressors, respectively, react with the C_κ and IVS probes, but not with the $V_\kappa 21$ or J_2 probes. The most plausible interpretation of these components is that they are unspliced derivatives of the κ mRNA precursors, which have undergone a cleavage at the left junction of the J–C intron but not at the right junction. The corresponding V-region cleavage products would not be in the poly(A)$^+$ fraction of nuclear RNA, and hence would not be detected in these experiments.

Some additional points of interest are shown in Fig. 3. First,

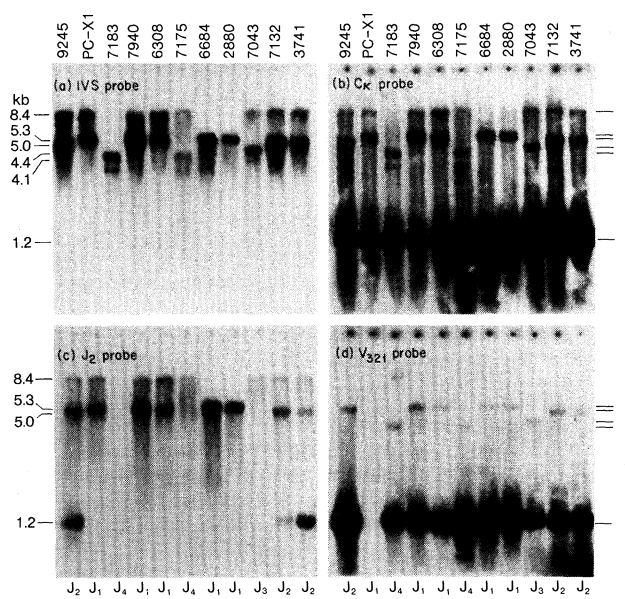


FIG. 2. Ig-related sequences in the nuclear RNAs of various plasmacytomas. Samples (10 μg) of poly(A)$^+$ nuclear RNA from selected plasmacytomas were fractionated on a pair of methylmercury-agarose gels, transferred to diazotized papers, and hybridized with ^{32}P-labeled IVS probe (*a*) or J_2 probe (*c*). After autoradiographic exposure the probes were melted off (10 min at 68°C in 99% formamide) and the RNAs were rehybridized with C_κ probe (*b*) and V_{321} probe (*d*). Tumor designation at top; expressed J segment (according to Fig. 1*b*) at bottom; component size at left. All tumors in this set except PC-X1 are members of the $V_\kappa 21$ group. PC-X1 was erroneously designated as PC4050 in a previous publication (7).

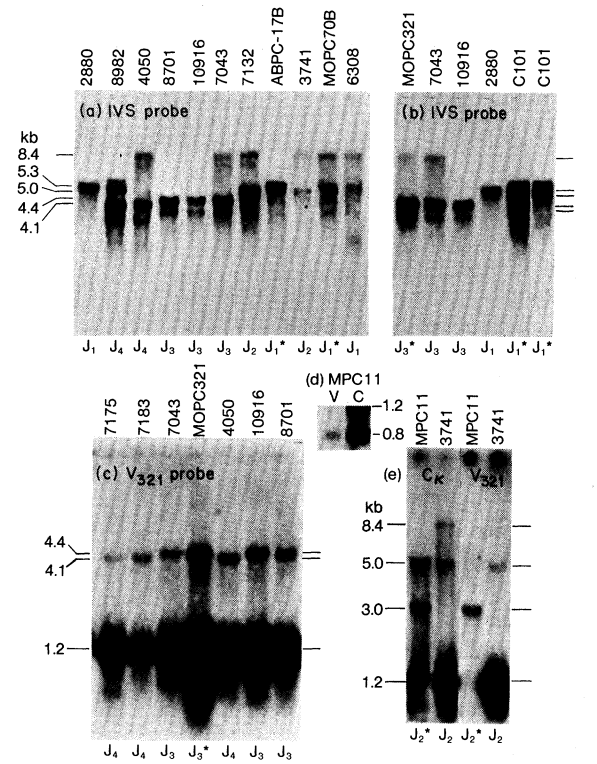


FIG. 3. Ig-related sequences in plasmacytoma nuclear RNAs. Samples of poly(A)$^+$ nuclear RNA (10 μg) (*a–c, e*) or poly(A)$^+$ cytoplasmic mRNA (1 μg) (*d*) from selected plasmacytomas, size fractionated and bound to diazotized paper as in Fig. 2, were hybridized with IVS probe (*a, b*), V_{321} probe (*c–e*) and C_κ probe (*d, e*). Tumor designation at top; expressed J segment at bottom (tumors of BALB/c origin are marked with asterisk); component size at left. The two lanes of C101 RNA in *b* represent two autoradiographic exposures of the same experiment.

there is no significant difference in the sizes of corresponding nuclear components in NZB and BALB/c plasmacytomas. Thus, the blot patterns observed with NZB J_1 expressors PC6308 and CP2880 have BALB/c counterparts in MOPC70 and ABPC-17B, respectively. The same is true for J_3 expressors PC7043 (NZB) and MOPC321 (BALB/c). Second, there are plasmacytomas such as PC8982, C101, and previously studied MPC-11, PC2960, and PC7210 (6, 7), that produce, in addition to the pre-mRNA for the expressed κ-chain, C_κ- and IVS-containing components that are significantly smaller than 8.4 kb. Note, for example, the components at about 5.2 and 6.0 kb in PC8982 cells and the ≈6-kb component in C101 cells. In some cases, e.g., in MPC-11 cells, these additional components are known to be processed into functional mRNAs (6). The MPC-11 nuclear components include a 5.0-kb species, which has the characteristics of a J_2-type pre-mRNA, and a 3-kb species, which appears to be the precursor of the 0.8-kb "fragment" mRNA that codes for the C_κ region and for an NH_2-terminal signal peptide, but not for a V region (18). Because this signal peptide is the same as that associated with the $V_\kappa 21$ genes (19, 20), the fragment mRNA reacts with the signal peptide encoding portion of the $V_\kappa 321$ probe as well as with the C_κ probe (Fig. 3*d*). The 3-kb nuclear component of MPC-11 cells reacts relatively strongly with the V_{321} probe (Fig. 3*e*), thus supporting the contention that it is the precursor of the fragment mRNA. These results are compatible with the recent finding of a second rearranged C_κ gene in MPC-11 cells, in which a $V_\kappa 21$ gene is located at a site about 1 kb downstream from the J_4 segment (J. G. Seidman and P. Leder, unpublished data). Processing of the 3-kb transcript presumably involves splicing between the C_κ region and the signal peptide-coding sequence and elimination of the element coding for the $V_\kappa 21$ region.

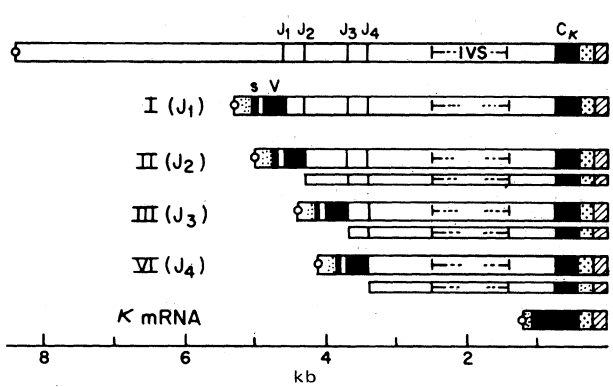


FIG. 4. Interpretation of the data of Figs. 2 and 3. The lengths and positions of various regions are obtained from sequence data (2, 3, 17). A poly(A) length of 0.2 kb is assumed; the length of the 5′ noncoding sequence is estimated by assuming that the total length of the mRNA is 1.2 kb. Solid areas, coding; stippled, noncoding; hatched, poly(A).

Several observations suggest that the genes that are remote from the C_κ region may be transcriptionally inactive. First, there is no detectable transcription of any $V_\kappa 21$ group gene in the non $V_\kappa 21$ group example PC-X1, which is clearly producing an 8.4-kb component and a J_1-type κ-mRNA, as evidenced by the reactivity of its RNA with the other probes. This applies to an analysis of poly(A)$^+$ nuclear RNA (Fig. 2) and an analysis of a 10-fold greater quantity of poly(A)$^-$ nuclear RNA (data not shown). A similar lack of reactivity was found when nuclear RNA from other non-$V_\kappa 21$ group tumors was examined with the $V_\kappa 21$ probe. Moreover, in tumors expressing one of the multiple (approximately six) genes of the $V_\kappa 21$ group, we are unable to detect discrete $V_\kappa 21$ transcripts other than the one associated with the expressed J segment [Fig. 3*c* and a similar analysis of poly(A)$^-$ nuclear RNA (not shown)]. Similarly, in our previous survey of $V_\kappa 21$ expressors with the ($V_\kappa 19$ + C_κ) probe (7) we did not observe any nuclear components that might be interpreted as transcripts of $V_\kappa 19$ genes. Because the level of detectability in such experiments is on the order of a few RNA molecules per cell (6), such negative observations should be biologically meaningful. Only in cases such as MPC-11 cells, in which V genes are translocated into both of the allelic C regions, is there any evidence for dual V region transcription. Although lack of transcription may sometimes be attributable to V gene deletion, this would presumably not be the case for V genes on an unrearranged chromosome or V genes located leftward of the expressed V gene. Thus, it seems reasonable to conclude that either the remote V_κ genes are transcriptionally silent or they produce transcripts that are too small or too unstable to be detected by the methods employed in this study.

A summary of data on 30 different plasmacytomas (Table 1) reveals 15 examples that produce the 8.4-kb transcript and 15 that do not. Our contention that this transcript emanates from the unrearranged C_κ allele would lead us to expect a correlation between the ability of a plasmacytoma to produce this large component and its possession of a germline-like J_κ–C_κ region. To verify whether such a correlation exists we used Southern blot analysis (14) to examine the genomic DNA of several plasmacytomas used in the above experiments. As a simple diagnostic for alteration in the germline context of the J–C region we focused on the 2.9-kb *Hin*dIII fragment that is specifically identified by the IVS probe (Fig. 1*b*). Because any

Table 1. Occurrence of the 8.4-kb J_κ–C_κ transcript among various plasmacytomas

Processing category (expressed J segment)	Presence of 8.4-kb transcript	No. of examples	Tumor designations
I (J_1)	+	7	PC2413, PC4999, PC6308, PC7461, PC7490, PC-X1, MOPC70
	−	6	PC1229, PC2880, PC6684, PC7210, PC7769, ABPC-17B
II (J_2)	+	3	PC3741, PC7132, PC9245
	−	3	PC2960, MPC11, C101
III (J_3)	+	3	PC3852, PC7043, MOPC321
	−	2	PC8701, PC10916
IV (J_4)	+	2	PC4050, PC7175
	−	4	PC2154, PC2485, PC7183, PC8982

Processing categories I–IV are defined by the nuclear components diagrammed in Fig. 4. Tumors prefixed by PC are of NZB origin; others are of BALB/c origin. All entries except PC3852, MPC11, and PC-X1 are in the $V_\kappa 21$ group. Plasmacytomas PC2960, PC7210, PC8982, MPC11, and C101 contain additional nuclear components, which in some (possibly all?) cases are related to secondary mRNAs containing C_κ sequences.

significant rearrangement in the J_κ region would necessarily alter the size of, or eliminate, this *Hin*dIII fragment, its presence in a particular sample of genomic DNA strongly indicates the presence of an unrearranged C_κ allele.

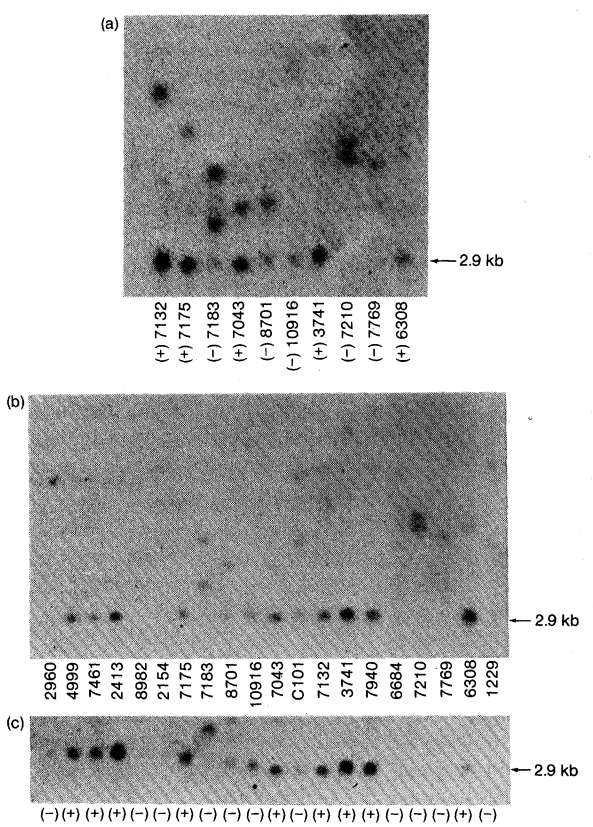


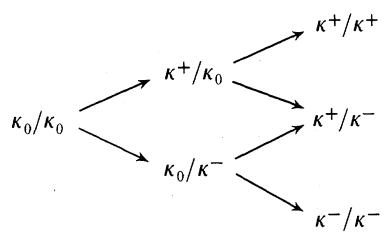
FIG. 5. Southern blots of *Hin*dIII digests of various plasmacytoma DNAs hybridized with the IVS probe. Blots *a*, *b*, and *c* are from three separate experiments. The tumors that produce the large 8.4-kb nuclear RNA are marked with a + symbol; those that do not are marked with a − symbol.

The results of this analysis (Fig. 5) show that a correlation does indeed exist. All nine of the plasmacytomas examined that produce the 8.4-kb transcript clearly contain an intact germline J_κ region in addition to the rearranged (expressed) J_κ region. Similarly, most of the tumors that do not produce the 8.4-kb component do not contain an unrearranged J_κ region, and in many cases (e.g., PC1229, PC7210, PC6684, and PC7183) exhibit two rearranged J_κ regions. There are a few tumors—*viz.* PC8701, PC10916, and C101—that do not produce the 8.4-kb component but nevertheless exhibit a relatively faint 2.9-kb *Hin*dIII band. In some cases—e.g., C101—we also observed two additional *Hin*dIII bands indicative of two rearranged J_κ regions and, therefore, we suspect that such relatively faint 2.9-kb bands might be derived from the DNA of extraneous cells that infiltrated these particular tumor samples.

DISCUSSION

The results of these studies enable us to distinguish four basic states of a κ locus in terms of organization, transcriptional activity, and processing potential of the RNA transcripts. (*i*) κ^+: when a DNA rearrangement (presumably a deletion) results in a V_κ gene being juxtaposed to one of the four functional J_κ segments, the assembled unit, including the associated C_κ gene, is transcribed and the transcript is processed into a functional light chain mRNA. (*ii*) κ_f: translocation of V_κ genes to sites other than J_κ segments can lead to the formation of transcriptionally active units involving the C_κ gene and to altered processing modes, as in the case of the MPC-11 fragment mRNA. (*iii*) κ_0: a C_κ gene that is still in the germline configuration is also transcribed, but in this case the 8.4-kb transcript is apparently not processed into any mRNA product. (*iv*) κ_x: certain types of rearrangements (deletions) in the C_κ region render it transcriptionally inactive. Conceivably, this type of deletion might even include loss of the C_κ gene itself. Of the 30 different plasmacytomas examined in our study, 15 (e.g., PC6308, PC3741, etc.) would be classified as κ^+/κ_0 and 10 (e.g., PC2880, PC7183, etc.) as κ^+/κ_x. The remaining 5 (MPC-11, PC2960, PC7210, PC8982, and C101) are most likely κ^+/κ_f, although two of these (PC8982 and C101) have not been sufficiently characterized to exclude κ^+/κ^+ as another possibility.

In terms of the allelic exclusion phenomenon, one might distinguish productive (i.e., κ^+) alleles and nonproductive (i.e., κ_0 and $\kappa^- \equiv \kappa_f$ or κ_x alleles. The substantial number of κ^+/κ_0 examples observed in our survey indicates that deletions at the two κ loci do not normally occur concurrently and that the generation of relevant genotypes might conform to the following two-step scheme:



The frequency of occurrence of the various genotypes depends on the rate at which deletions occur, which will determine the relative number of examples with transitions at one or both κ alleles, and on the relative probabilities of $\kappa_0 \rightarrow \kappa^+$ and $\kappa_0 \rightarrow \kappa^-$ transitions. There may be other contributing factors, such as a diminished likelihood that subsequent transitions in κ^+/κ_0 genotypes would be detected in clonally expanding populations of immunoglobulin-producing cells, or selective processes that

might eliminate certain genotypes from the population pool—e.g., selection against cells bearing κ^+/κ^+ genotypes. The results of the present study, which are necessarily restricted to genotypes consisting of at least one κ^+ allele, indicate essentially equal proportions of κ^+/κ_0 and κ^+/κ^- genotypes and few, if any, κ^+/κ^+ genotypes. Although the multiplicity of unknown factors enumerated above precludes a unique explanation for our data, some predications can be made if we consider that the transition rates are high enough to generate equivalent numbers of cells with deletions in one and both chromosomes. In this case our data could best be explained by a model with similar probabilities for $\kappa_0 \rightarrow \kappa^+$ and $\kappa_0 \rightarrow \kappa^-$ transitions—i.e., with a limited number of possibilities for nonproductive deletions. For a further evaluation of this model it would be useful to know the frequency of occurrence of κ_0/κ^- genotypes in cells producing λ light chains.

The formation of a productive immunoglobulin gene may be viewed abstractly as the joining of two regions that mutually provide the necessary and sufficient elements for expression. One element, the capacity to generate stable transcripts of significant size, seems to require participation of the C region. In plasmacytoma cells the C region locus is transcriptionally active even when in the germline configuration, whereas there is no detectable transcription at unrearranged V region loci. This may be due to the C region being in a chromosomal zone of superior template activity or, alternatively, to a contribution of proper termination and polyadenylylation signals required for transcript stability. For the expressed κ gene, initiation of transcription occurs at a point upstream from the signal peptide coding sequence and all 5′ untranslated leader segments. The exact location of this initiation point is still unknown, although if we assume that the primary transcript is represented by the largest detectable poly(A)$^+$ nuclear component containing V coding sequences, our best size estimates (Fig. 4) would place it about 0.25 kb upstream from the signal peptide coding sequence. In the case of MOPC321 cells the integrity of at least 8 kb of germline DNA upstream of the V_{321} gene is maintained during the V–J joining process (10). Thus, the signal for transcriptional initiation is presumably associated with the unrearranged V gene element, although apparently in an inactive state. The existence of a widely spaced upstream leader segment, as proposed for MOPC21 myelomas (5), is not indicated for the $V_\kappa 21$ genes or for some non-$V_\kappa 21$ genes such as those expressed in MPC-11, PC3852, and PC-X1 tumors. For MPC-11 cells, the 5.0-kb component characteristic of J_2 expressors is the largest detectable component in 7.5-min-labeled total nuclear RNA (6), an observation that supports the notion that it is, in fact, a primary transcript. The nature of the initiation point for the 8.4-kb transcript of the germline κ allele is still unknown. It could conceivably belong to an unidentified member of the V_κ gene family, or it could be a unique site with promoter activity.

Another necessary element for gene expression, processing competence, apparently has important structural requirements that are satisfied only in transcripts of appropriately rearranged C regions. There are no detectable processing products of the 8.4-kb germline C region transcript in spite of the fact that both C_κ and J_κ splice junctions are contained in it. The possibility that the 3.4-, 3.7-, and 4.3-kb components that lack V region sequences are such products is remote because the size of these components is directly related to the particular J segment that is expressed. The implication of subtle structural (conformational?) requirements for the processing of κ mRNA precursors is also indicated by the fact that J–C splicing occurs only with the particular J segment that is contiguous to V, even though the relevant pre-mRNA may contain additional J segments, as is the case in J_1, J_2, and J_3 expressors. Of course, both the V and the C regions contribute elements that are essential for the proper functioning of the mature mRNA.

A final point that deserves mention is the existence of possible processing intermediates formed when cleavage at the 5′ boundary of the J–C intron has occurred prior to cleavage at the 3′ boundary. If this interpretation is correct, it would imply that the cleavages at the intron boundaries do not always occur concurrently. Because such intermediates are never observed in J_1 expressors, are occasionally observed in J_2 expressors, and are more frequently observed in J_3 and J_4 expressors, the relative synchrony of the cleavage events would appear to be dependent on the structure of the transcript. Because the primary sequences surrounding the four J_κ intron junctions are identical (2, 3), this may be another example of the influence of conformation on processing competence.

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