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Identification of a Germ Line Transcript from the Unrearranged Kappa Gene in Human B Cells

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A novel kappa immunoglobulin-hybridizing mRNA in cell lines derived from human B cells arrested at several stages of development has been identified. Hybridization studies demonstrate that this 1.5-kilobase mRNA species is the spliced product of a precursor germ line transcript initiating upstream of the unrearranged J_κ locus.

Both mouse and human kappa (κ) light-chain expression requires that one of the numerous variable (V_κ)-region genes rearrange to one of four (mouse) or five (human) joining (J_κ) gene segments situated 2.6 to 4.2 kilobases (kb) upstream of the kappa constant region (C_κ) (2, 8, 25). When properly assembled, transcriptional control elements located upstream of the rearranged V_κ are activated by the enhancer sequence located in the J_κ-C_κ intron (4, 5). Primary transcripts are subsequently processed by splicing the signal exon to the VJ_κ exon and the VJ_κ exon to the C_κ exon, yielding a functional 1.2-kb mRNA (14, 21). In addition to a transcript from a rearranged kappa locus, an 8.4-kb nuclear-RNA transcript from the unrearranged or germ line (κ^0) allele was identified in mouse plasmacytoma cells and pre-B cells (17).

It has been proposed that transcription of the κ^0 allele might serve to facilitate subsequent recombinatorial events at this locus (2, 27). This model is substantiated by studies that have identified heavy-chain mu (C_μ), DJ_H-C_μ, and variable-gene-segment (V_H) transcripts (1, 9, 18, 29, 30) at early stages of B-cell development. A correlation was also found between heavy-chain constant-region isotype germ line transcription and the induction of cell switching to a given isotype (13, 24). Thus, it appears that transcription from unrearranged exons may play an important role in the ordered recombination observed in the development of murine B cells. Because of the similarities of organization and rearrangement found between the murine and human κ loci, we postulate that germ line transcripts may be expressed prior to genetic recombination during human-B-cell development.

A novel transcript from the unrearranged κ allele in human B cells. Transcription of the unrearranged κ^0 allele is seen even in murine plasmacytoma cells in which the other allele has either functionally rearranged (κ^+) or nonfunctionally rearranged (κ^-) (17). On the basis of this observation, human cell lines with the allele status $\kappa^0\kappa^+$ or $\kappa^0\kappa^-$ were screened for potential κ^0 RNA expression (Table 1). Northern (RNA) blots (3) of total RNA (6) isolated from the selected cell lines were hybridized with the HuC_κ probe (Fig. 1). As a control, cell lines in which both κ alleles had rearranged or had been deleted (κ^d) were also analyzed. These control cell lines, SU-DHL 16 (22) and IM9 (ATCC CCL 159) (Fig. 1 and Table 1), contained only a 1.2-kb mRNA, which is the expected size for the productive (κ^+) κ mRNA. In contrast, the functional κ-producing cell line SU-DHL 4 ($\kappa^0\kappa^+$) (22) contained the 1.2-kb mRNA as expected, but it also produced a 1.5-kb mRNA. Similarly, the nonfunctional κ-producing cell line HS Sultan ($\kappa^0\kappa^-$) (ATCC CRL 1484) produced a 1.5-kb mRNA as well as a truncated 0.8-kb mRNA.

BLIN-1 is a human pre-B-cell line that spontaneously rearranges κ light-chain genes in culture (28). Several subclones were screened for potential κ^0 mRNA. When the BLIN-1 subclones C2 and 0B5 ($\kappa^0\kappa^0$) were analyzed, a 1.5- and a 1.2-kb mRNA were detected in the poly(A)$^+$ RNA fraction (Fig. 1). The 1.5-kb mRNA species was not detected in the $\kappa^+\kappa^d$ subclone 1E8 (Fig. 1). A survey of cell lines derived from a variety of B cells arrested at several stages of development showed that the expression of the 1.5-kb mRNA species correlated with the presence of a κ^0 allele (Table 1).

Induction of κ^0 transcription in human pre-B cells. The pre-B-cell lines LAZ 221 ($\kappa^0\kappa^0$) (12) and Nalm 16 ($\kappa^0\kappa^0$) (11) do not express κ transcripts. Since it has been noted that B-cell mitogens such as lipopolysaccharide (LPS) have been used to induce κ^0 transcription in the murine cell lines 3-1

TABLE 1. Occurrence of the 1.5-kb mRNA C_κ transcript in various human B cells

Cell line	Allele status	Source[a]	RNA species[b]	
			1.2 kb	1.5 kb
SU-DHL 4	$\kappa^0\kappa^+$	DHL	+	+
HS Sultan	$\kappa^0\kappa^-$	PC	(+)	+
S.N.[c]	$\kappa^0\kappa^+$	CLL	+	+
S.R.[c]	$\kappa^0\kappa^+$	CLL	+	+
LAZ 221[d]	$\kappa^0\kappa^0$	ALL	(+)	+
Nalm 16[d]	$\kappa^0\kappa^0$	ALL	(+)	+
BLIN-1 (0B5)	$\kappa^0\kappa^0$	ALL	(+)	+
BLIN-1 (C2)	$\kappa^0\kappa^0$	ALL	(+)	+
BLIN-1 (1E8)	$\kappa^d\kappa^+$	ALL	+	−
WET	$\kappa^-\kappa^+$	Lymph	+	−
SU-DHL 16	$\kappa^d\kappa^+$	DHL	+	−
IM9	$\kappa^d\kappa^+$	CML	+	−
Nalm 6[d]	$\kappa^d\kappa^d$	ALL	−	−
Ramos	$\kappa^d\kappa^d$	Lymph	−	−

[a] DHL, Diffuse histocytic lymphoma; PC, plasmacytoma; CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia; Lymph, lymphoma; CML, chronic myelogenous leukemia.
[b] +, Present; −, absent; (+), faintly present.
[c] Lymphoid fraction of Ficoll-Hypaque gradient from patients with chronic lymphocytic leukemia.
[d] Induced with LPS.

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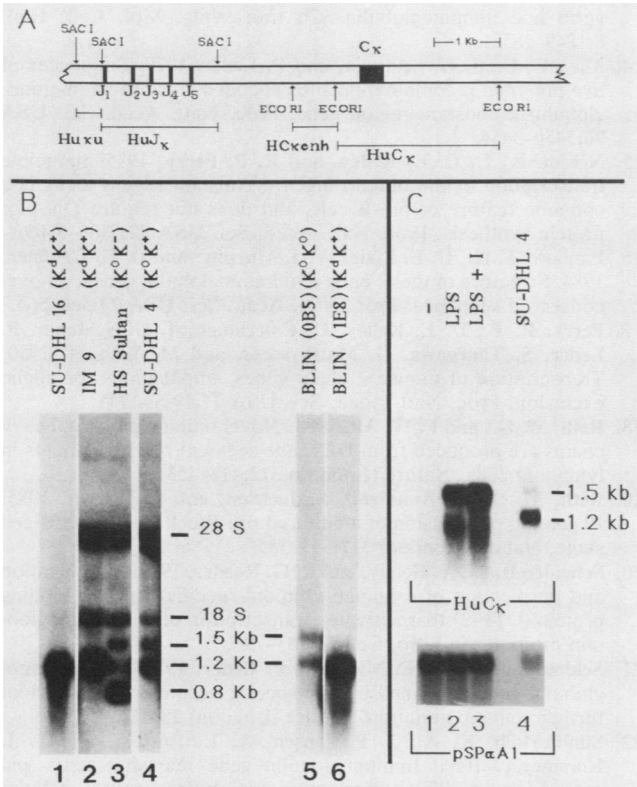

FIG. 1. (A) Restriction map of the κ germ line locus indicating location of fragments that were isolated for specific κ-region probes. (B) Expression of κ RNA in human B cells. Total RNA (20 μg; lanes 1 through 4) or poly(A)$^+$ RNA (4 μg; lanes 5 and 6) was analyzed by Northern blot for κ mRNA by using the $C_κ$-specific probe $HuC_κ$. Sizes of RNAs were based on the mobility of an RNA ladder (Bethesda Research Laboratories, Inc.). Bands at 28S and 18S are from cross-hybridization of the probe with rRNAs. (C) Induction of LAZ 221 with LPS (15 μg/ml) or LPS plus an ionophore (I) (1 μg/ml). Cytoplasmic poly(A)$^+$ RNA (5 μg) was analyzed by Northern blot for κ RNA by hybridization with $HuC_κ$. Also shown is a reprobe of the Northern blot with an actin-specific probe, pSPα A1, to demonstrate equal loading of LAZ 221 RNA in each lane.

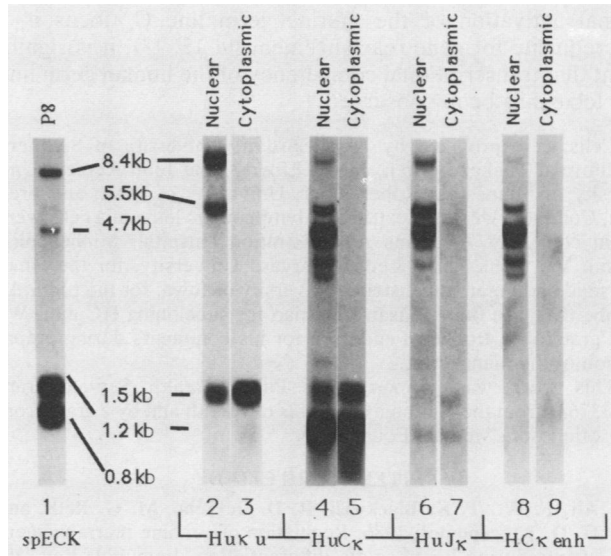

FIG. 2. Nuclear poly(A)$^+$ RNA (4 μg) and cytoplasmic poly(A)$^+$ (2 μg) were analyzed by Northern blot for germ line κ expression. Lane 1, Nuclear RNA from the Abelson murine leukemia virus-transformed cell line P8 (19) probed with the murine κ-specific probe $EC_κ$ (26); lanes 2 and 3, Northern blot of RNA from human cell lines SU-DHL 4 ($κ^+κ^0$) probed with Huκu; lanes 4 and 5, reprobe of the Northern blot with $HuC_κ$; lanes 6 and 7, reprobe with $HuJ_κ$; lanes 8 and 9, reprobe with $HC_κ$enh.

($κ^0κ^0$) and 1-8 ($κ^0κ^0$) (15), attempts were made to induce germ line κ expression in the human cell lines LAZ 221 and Nalm 16. The addition of LPS (15 μg/ml) alone for 16 h or LPS plus the ionophore A23187 (1 μg/ml) for 8 h resulted in the induction of a 1.5- and a 1.2-kb mRNA species from LAZ 221 (Fig. 1) and Nalm 16 that hybridized to $HuC_κ$. The presence of the 1.5-kb mRNA in cell lines with both alleles in germ line configuration confirms that the 1.5-kb mRNA was expressed from the unrearranged κ locus.

Nuclear $κ^0$ transcripts. To identify the nuclear $κ^0$ transcript, a 560-base-pair *Sac*I fragment (Huκu) containing sequences just upstream of the $J_κ$ region was excised from the 12-kb *Bam*HI Huκ1 fragment (7) (Fig. 1). Thus, if a κ allele had undergone rearrangement such that a $V_κ$ had fused to a $J_κ$, this *Sac*I fragment would be deleted and only transcripts derived from the $κ^0$ allele would be detected with this probe. Nuclear and cytoplasmic poly(A)$^+$ RNAs were isolated from SU-DHL 4 ($κ^+κ^0$), and the subsequent Northern blot was probed with Huκu. An 8.5- and a 5.5-kb nuclear transcript plus the 1.5-kb mRNA hybridized with this upstream probe, while the 1.2-kb mRNA derived from the $κ^+$ allele did not (Fig. 2). Subsequent probing with $HuC_κ$ confirmed that the 8.5-, 5.5-, and 1.5-kb RNAs were indeed κ immunoglobulin-specific. The $κ^+$ precursor RNA, several splicing intermediates, and the 1.2-kb $κ^+$ mRNA were also identified when hybridized with $HuC_κ$. All RNA species also hybridized to the $J_κ$ region probe $HuJ_κ$, but only the nuclear-specific RNA hybridized with the intron region probe $HC_κ$enh. This indicates that the 1.2- and the 1.5-kb mRNAs are the final splice products for the $κ^+$ and $κ^0$ alleles, respectively. The steady-state content of the human nuclear $κ^0$ transcript(s) appears lower than that of the $κ^+$ transcript, which correlates with what has been observed for mouse hybridomas and plasmacytomas (9).

The relative sizes of the nuclear transcripts identified in mice and humans are strikingly similar, suggesting that there is an initiation site 3 to 4 kb upstream of the $J_κ$ region (27). No DNA clones or nucleotide sequences are currently available for the region of initiation of the human $κ^0$ transcript. Therefore, we have not yet precisely mapped the start site, nor have we searched for structural similarities or control elements (16, 20, 27) which may be conserved. The 5.5-kb nuclear species could be either a second $κ^0$ primary-RNA transcript with initiation starting about 1 kb upstream of $J_κ1$, as observed in mice (D. J. Martin, unpublished data), or a splicing intermediate of the 8.5-kb $κ^0$ transcript. Since 1.2-kb mRNAs are also seen in the pre-B cell lines BLIN-1 (0B5) and LPS-stimulated LAZ 221 (Fig. 1), there is a possibility that this product is also derived from the unrearranged κ locus. This would be similar to the situation with mice, in which there are multiple processed germ line transcripts (Fig. 2, lane 1) (10, 15). Alternatively, the 1.2-kb mRNA could represent transcription from a subpopulation of cells that have undergone rearrangement at the kappa locus.

The evolutionary maintenance of κ germ line transcription strongly suggests that it plays an important role in B-cell development. Since it has been postulated that transcrip-

tional activation of the murine germ line C_κ locus is a prerequisite for gene rearrangement (9, 12, 13), it is significant that transcriptional competency of the human germ line C_κ locus has been conserved.

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