

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}), $DJ_{H}C_{\mu}$, 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 histiocytic 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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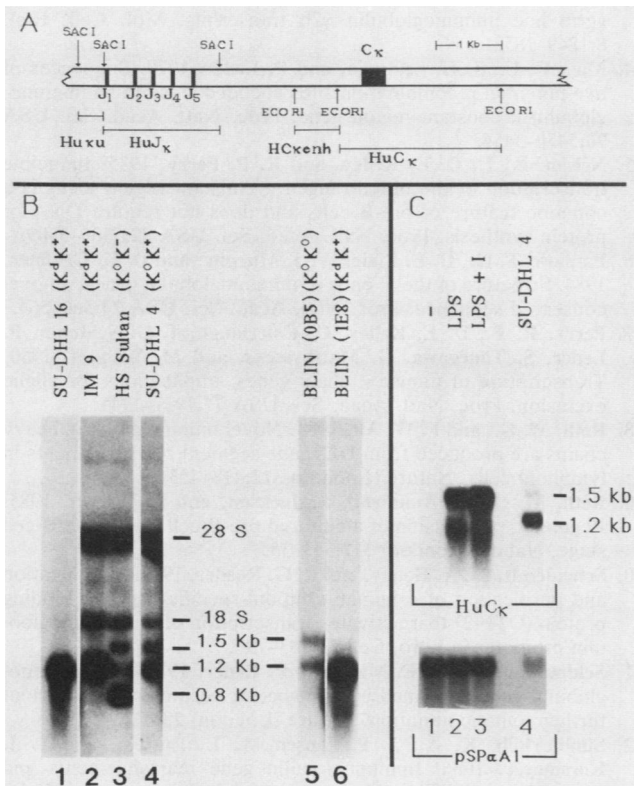


FIG. 1. (A) Restriction map of the κ germ line locus indicating location of fragments that were isolated for specific κ -region probes. (B) Expression of κ RNA in human B cells. Total RNA (20 μ g; lanes 1 through 4) or poly(A)⁺ RNA (4 μ g; lanes 5 and 6) was analyzed by Northern blot for κ mRNA by using 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 μ g/ml) or LPS plus an ionophore (I) (1 μ g/ml). Cytoplasmic poly(A)⁺ RNA (5 μ g) 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, pSP α A1, to demonstrate equal loading of LAZ 221 RNA in each lane.

($\kappa^0\kappa^0$) and 1-8 ($\kappa^0\kappa^0$) (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 μ g/ml) alone for 16 h or LPS plus the ionophore A23187 (1 μ g/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 *SacI* fragment ($HuKu$) containing sequences just upstream of the J_{κ} region was excised from the 12-kb *BamHI* $Hu\kappa 1$ fragment (7) (Fig. 1). Thus, if a κ allele had undergone rearrangement such that a V_{κ} had fused to a J_{κ} , this *SacI* 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 ($\kappa^+\kappa^0$), and the subsequent Northern blot was probed with $HuKu$. 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}

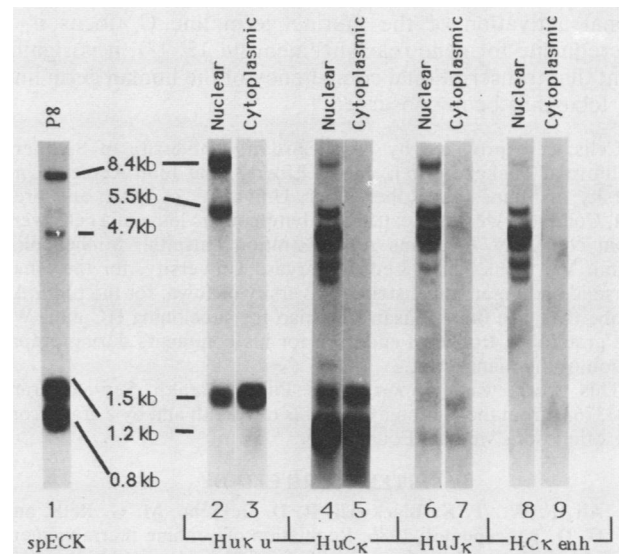


FIG. 2. Nuclear poly(A)⁺ RNA (4 μ g) and cytoplasmic poly(A)⁺ (2 μ g) were analyzed by Northern blot for germ line κ expression. Lane 1, Nuclear RNA from the Abelson murine leukemia virus-transformed cell line P8 (19) probed with the murine κ -specific probe EC_{κ} (26); lanes 2 and 3, Northern blot of RNA from human cell lines SU-DHL 4 ($\kappa^+\kappa^0$) probed with $HuKu$; 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 $HC_{\kappa}enh$.

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 $HC_{\kappa}enh$. 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 $J_{\kappa}1$, 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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LITERATURE CITED

- Alt, F. W., T. K. Blackwell, R. D. DePinho, M. G. Reth, and G. D. Yancopoulos. 1986. Regulation of genome rearrangement events during lymphocytic differentiation. *Immunol. Rev.* **89**: 5–30.
- Alt, F. W., T. K. Blackwell, and G. D. Yancopoulos. 1987. Development of the primary antibody repertoire. *Science* **238**: 1079–1087.
- Alwine, J. C., D. J. Kemp, B. A. Parker, J. Reiser, J. Renart, G. R. Stark, and G. M. Wahl. 1979. Detection of specific RNAs or specific fragments of DNA by fractionation in gels and transfer to diazobenzoyloxymethyl paper. *Methods Enzymol.* **68**:220–242.
- Atchison, M. L., and R. P. Perry. 1987. The role of the κ enhancer and its binding factor NF- κ B in the developmental regulation of κ gene expression. *Cell* **48**:121–128.
- Calame, K. L. 1985. Mechanism that regulates immunoglobulin gene expression. *Annu. Rev. Immunol.* **3**:159–195.
- Chomczynski, P., and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159.
- Hieter, P. A., J. V. Maizel, Jr., and P. Leder. 1982. Evolution of human immunoglobulin κ J region genes. *J. Biol. Chem.* **257**: 1516–1522.
- Hieter, P. A., E. E. Max, J. G. Seidman, J. V. Maizel, Jr., and P. Leder. 1980. Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments. *Cell* **22**:197–207.
- Kelley, D. E., and R. P. Perry. 1986. Transcriptional regulation of immunoglobulin V genes. *Nucleic Acids Res.* **14**:5431–5447.
- Kelley, D. E., B. A. Pollok, M. L. Atchison, and R. P. Perry. 1988. The coupling between enhancer activity and hypomethylation of κ immunoglobulin genes is developmentally regulated. *Mol. Cell. Biol.* **8**:930–937.
- Korsmeyer, S. J., A. Arnold, A. Bakhshi, J. V. Ravetch, U. Siebenlist, P. A. Hieter, S. O. Sharrow, T. W. LeBien, J. H. Kersey, D. G. Poplack, P. Leder, and T. A. Waldman. 1983. Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemias of T cell and B cell precursor origins. *J. Clin. Invest.* **71**:301–313.
- Lazarus, H., E. F. Barell, A. Krishan, D. M. Livingston, K. Harris, S. F. Schlossman, and L. Chess. 1978. Characterization of a unique cell line (LAZ 221) from human acute lymphocytic ("Null") leukemia. *Cancer Res.* **38**:1362–1366.
- Lutzker, S., and F. W. Alt. 1988. Structure and expression of germ line immunoglobulin γ 2b transcripts. *Mol. Cell. Biol.* **8**:1849–1852.
- Max, E. E., J. G. Seidman, and P. Leder. 1979. Sequences of five potential recombination sites encoded close to an immunoglobulin κ constant region gene. *Proc. Natl. Acad. Sci. USA* **76**:3450–3454.
- Nelson, K. J., D. E. Kelley, and R. P. Perry. 1985. Inducible transcription of the unrearranged κ constant region locus is a common feature of pre-B cells and does not require DNA or protein synthesis. *Proc. Natl. Acad. Sci. USA* **82**:5305–5309.
- Parslow, T. G., D. L. Blair, W. J. Murphy, and D. K. Granner. 1984. Structure of the 5' ends of immunoglobulin genes: a novel conserved sequence. *Proc. Natl. Acad. Sci. USA* **74**:560–564.
- Perry, R. P., D. E. Kelley, C. Coleclough, J. G. Seidman, P. Leder, S. Tonegawa, G. Matthysens, and M. Weigert. 1980. Transcription of mouse κ chain genes: implications for allelic exclusion. *Proc. Natl. Acad. Sci. USA* **77**:1937–1941.
- Reth, M. G., and F. W. Alt. 1984. Novel immunoglobulin heavy chains are produced from DJ $_H$ gene segment rearrangements in lymphoid cells. *Nature (London)* **312**:418–423.
- Reth, M. G., P. Ammirati, S. Jackson, and F. W. Alt. 1985. Regulated progression of a cultured pre-B cell line to the B-cell stage. *Nature (London)* **317**:353–355.
- Scheidereit, C., A. Heguy, and R. G. Roeder. 1987. Identification and purification of a human lymphoid-specific octamer binding protein (OTF-2) that activates transcription of an immunoglobulin promoter in vitro. *Cell* **51**:783–793.
- Seidman, J. G., E. E. Max, and P. Leder. 1979. A κ -immunoglobulin gene is formed by site-specific recombination without further somatic mutation. *Nature (London)* **280**:370–375.
- Siminovitch, K. A., J. P. Jensen, A. L. Epstein, and S. J. Korsmeyer. 1986. Immunoglobulin gene rearrangements and expression in diffuse histiocytic lymphomas reveal cellular lineage, molecular defects, and sites of chromosomal translocation. *Blood* **67**:391–397.
- Solomon, L. R., and P. A. Rubenstein. 1987. Studies on the role of actin's N $^{\gamma}$ -methylhistidine using oligonucleotide-directed site specific mutagenesis. *J. Biol. Chem.* **262**:11382–11388.
- Stavnezer, J., G. Radcliffe, Y.-D. Lin, J. Nietupski, L. Berggren, R. Sitia, and E. Severinson. 1988. Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes. *Proc. Natl. Acad. Sci. USA* **85**:7704–7708.
- Tonegawa, S. 1983. Somatic generation of antibody diversity. *Nature (London)* **302**:575–581.
- Van Ness, B. G., C. Coleclough, R. P. Perry, and M. Weigert. 1982. DNA between variable and joining gene segments of immunoglobulin κ light chain is frequently retained in cells that rearrange the κ locus. *Proc. Natl. Acad. Sci. USA* **79**:262–266.
- Van Ness, B. G., M. Weigert, C. Coleclough, E. L. Mather, D. E. Kelley, and R. P. Perry. 1981. Transcription of the unrearranged mouse C_{κ} locus: sequence of the initiation region and comparison of activity with a rearranged $V_{\kappa}-C_{\kappa}$ gene. *Cell* **27**:593–602.
- Wormann, B., J. M. Anderson, J. A. Liberty, K. Gajl-Peczalska, R. D. Brunning, T. L. Silberman, D. C. Arthur, and T. W. LeBien. 1989. Establishment of a leukemic cell model for studying human pre-B to B cell differentiation. *J. Immunol.* **142**:110–117.
- Yancopoulos, G. D., and F. W. Alt. 1985. Developmentally controlled and tissue-specific expression of unrearranged V_H gene segments. *Cell* **40**:271–281.
- Yancopoulos, G. D., and F. W. Alt. 1986. Regulation of the assembly and expression of variable-region genes. *Annu. Rev. Immunol.* **4**:339–368.