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Structure and Expression of Germ Line Immunoglobulin γ2b Transcripts

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We have isolated a cDNA copy of a truncated C_γ2b transcript produced by Abelson murine leukemia virus transformants that spontaneously switch from μ to γ2b. The initiation site of this transcript was 2 kilobases 5′ to the γ2b switch recombination region, demonstrating its germ line origin. Nucleotide sequence analyses suggest that this transcript does not encode a protein. Expression of germ line γ2b transcripts in Abelson murine leukemia virus transformants and in normal spleen cells correlated with endogenous γ2b class switch activity.

The heavy-chain constant region (C_H) determines the effector functions of the antibody molecule, such as complement fixation, transversing the placenta, and binding to Fc receptors. There are eight classes of immunoglobulins in the mouse, each determined by a distinct C_H gene. The C_H gene order is 5′-variable-region gene C_μ-C_δ-C_γ3-C_γ1-C_γ2b-C_γ2a-C_ε-C_α-3′; this locus spans over 200 kilobases (9). Initially, the assembled heavy-chain variable-region (V_H) gene lies upstream of the C_μ constant region; this configuration leads to μ production. The production of heavy chains encoded by downstream C_H genes usually requires a recombination event which juxtaposes the assembled variable-region gene to a new constant region and deletes intervening constant regions (9). The recombination event generally occurs within regions upstream of each C_H gene (except C_δ), referred to as switch (S) regions (6, 8, 9). A specific mechanism to recombine S regions appears to exist in B lineage cells (7). The frequent occurrence of recombination events to the same S region on the two alleles of plasmacytomas, immunocytomas, and normal B cells suggests that class switching may not be a random process (4, 11, 13). I.29 B lymphoma cells produce C_α-, C_ε- and C_γ2a-hybridizing transcripts prior to switching to these C_H genes (10), and Abelson murine leukemia virus (A-MuLV)-transformed pre-B cell lines express C_γ2b-hybridizing transcripts prior to switching to γ2b (14), leading to the idea that directed switching could be achieved by modulating the accessibility of a given S region to a common S recombinase (10, 14).

Structure of germ line γ2b transcripts. The pre-B cell line 300-18P switches spontaneously from μ to γ2b production in culture and produces transcripts that hybridize to a C_γ2b probe but not to probes specific for either of the two rearranged variable-region genes (14). These transcripts are approximately 3.6 and 1.7 kilobases, slightly smaller than the membrane and secreted forms of authentic V_H-containing γ2b mRNA (Fig. 1A; compare RNA from 300-18P with that from 300-18P-6, a γ2b protein-producing subclone), but approximately 300 base pairs (bp) longer than the predicted size of a transcript containing only a γ2b constant region. To identify sequences that contribute the additional 300 bp, total RNAs from 300-18P and from the γ2b protein-producing subclone were assayed for hybridization to probes representing genomic sequences located upstream of S_γ2b (Fig. 1C). The truncated γ2b transcripts hybridized to both the *Sac*I-*Sac*I (Fig. 1B, lane 300-18P) and *Xba*I-*Sac*I probes from the region upstream of S_γ2b but not to either the *Bam*HI-*Xba*I or *Hin*cII-*Hin*cII probes (not shown), localizing the hybridizing sequences to a region between the *Xba*I and *Hin*cII sites. This portion of the mRNA will be referred to as I_γ2b, because it was derived from the intervening region between C_γ1 and C_γ2b. A cDNA clone derived from the truncated γ2b mRNA was isolated. Comparison of the nucleotide sequence of the 5′ end of this cDNA to genomic sequences from I_γ2b and from the C_H1 domain of C_γ2b showed that the I_γ2b sequence was spliced directly onto the C_H1 domain (Fig. 1D). This splicing event appeared to employ a consensus splice donor site located where the I_γ2b sequence diverged from that of the cDNA and the normal splice acceptor site 5′ of exon 1 of C_γ2b; the spliced product did not contain S region sequences.

To map the potential transcription initiation sites of the truncated γ2b transcripts, a uniformly labeled single-stranded probe prepared from the *Bam*HI-*Hin*cII fragment upstream of S_γ2b (Fig. 1C and 2C) was used in S1 nuclease protection analyses. This probe generated the same seven S1-protected fragments, with sizes ranging from 260 to 400 bp, in RNA from three A-MuLV transformants (Fig. 2A). In addition, primer extension by an end-labeled 238-bp *Bam*HI-*Xho*I fragment from a γ2b cDNA (Fig. 2C) resulted in seven primer extension products that corresponded to the seven S1-protected fragments (Fig. 2B), confirming that transcription initiated heterogeneously within a 140-bp region. The transcription initiation region lacked a well-defined TATAA motif (Fig. 3), consistent with the sequence of other promoter regions that have heterogeneous initiation sites (2). Clearly, the truncated γ2b transcripts represented germ line γ2b transcripts, because they were initiated in a region immediately upstream of S_γ2b; the initiation region would be deleted from cells that had undergone γ2b class switching.

There were multiple stop codons in all three reading frames of the germ line γ2b RNA sequence, with three in the reading frame that would allow translation of the γ2b constant region; there were no ATG initiation codons in this reading frame (Fig. 3). Previously, no γ2b-related proteins were detectable in A-MuLV transformants that produce

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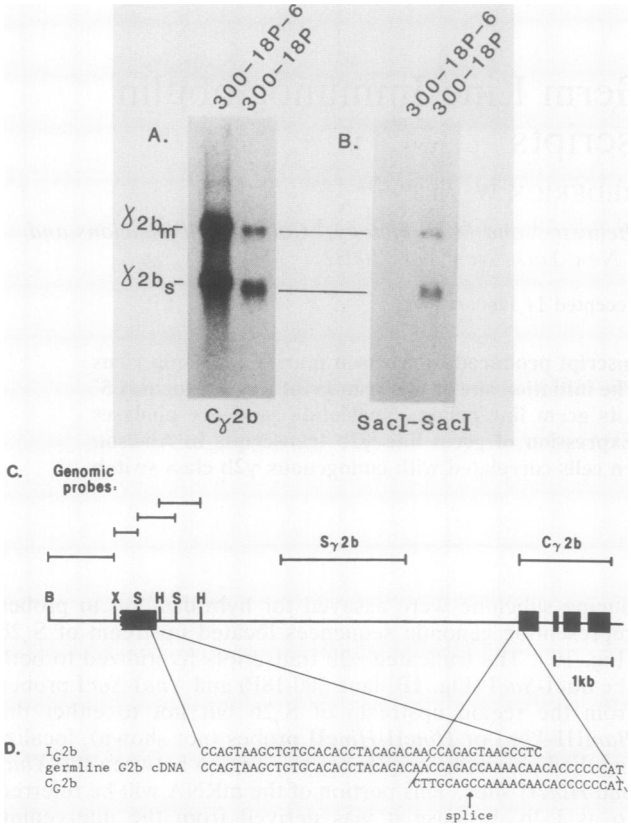

FIG. 1. Characterization of truncated γ2b transcripts. Total RNAs from the indicated cell lines were assayed by Northern (RNA) blotting as previously described (14). (A) Hybridization with a C_γ2b probe. (B) Hybridization with a *Sac*I-*Sac*I probe (panel C) derived from the region immediately upstream of S_γ2b. (C) Map of the γ2b heavy-chain locus with genomic probes indicated. Restriction endonuclease sites: B, *Bam*HI; X, *Xba*I; *S*, *Sac*I; *H*, *Hin*cII. (D) Nucleotide sequence of the 5′ end of the truncated γ2b cDNA isolated from the 3-1 A-MuLV transformant. The nucleotide sequence of the I_γ2b (I_G2b) region upstream of S_γ2b was determined (see Fig. 3); the portion of the sequence homologous to the germ line γ2b (G2b) cDNA is shown with the published sequence of the C_H1 domain of C_γ2b (C_G2b) and its immediate 5′-flanking sequence (12). kb, Kilobase; γ2b$_m$, γ2b membrane; γ2b$_s$, γ2b secreted.

germ line γ2b transcripts (1). Thus, with regard to structure and lack of obvious protein-encoding capacity, germ line γ2b transcripts resemble sterile μ transcripts that initiate heterogeneously within the μ heavy-chain enhancer (5). The heavy-chain enhancer is composed of a number of DNA motifs that contribute to the transcriptional enhancing activity of this region (3). None of these motifs were found in the germ line γ2b initiation region, suggesting that the expression of germ line γ2b transcripts is regulated differently from that of sterile μ transcripts.

Expression of germ line γ2b transcripts. To examine the expression of both normal and germ line γ2b transcripts in the various cells, an S1 nuclease protection assay was performed (Fig. 4B). Hybridization of the S1 probe to the homologous germ line transcript will completely protect the portion derived from the germ line γ2b transcript, to yield a 162-bp S1-nuclease-resistant fragment. However, hybridization to transcripts that contain the C_γ2b region linked to sequences other than the I_γ2b exon, such as VDJ-C_γ2b transcripts, will protect only the C_γ2b portion of the probe, to yield a 138-bp protected fragment. Levels of germ line γ2b transcripts varied among the A-MuLV-transformed lines assayed (Fig. 4A). However, all clones and subclones which spontaneously undergo μ-to-γ2b class switches (as indicated by the presence of VDJ-C_γ2b transcripts in the population that resulted in a 138-bp protected fragment [Fig. 4A] and confirmed by direct DNA and protein analyses in subclones [data not shown]) produced detectable levels of the germ line transcripts (e.g., 18-81A20, 300-18P-15, S11γ tk−), whereas 38B9γtk−, an A-MuLV transformant that does not undergo significant levels of endogenous switching but recombines transfected S sequences which are transcribed from a viral long terminal repeat (7), did not show detectable germ line expression. The γ2b protein-producing subclone 300-18P-6 also did not produce detectable levels of germ line γ2b transcripts and, correspondingly, did not switch at a detectable frequency on its nonproductive allele (data not shown).

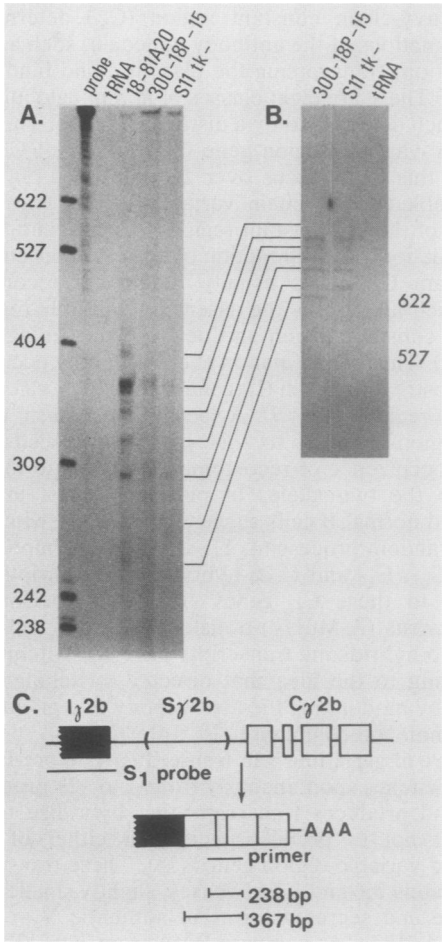

FIG. 2. Initiation sites of truncated γ2b transcripts. (A) An S1 nuclease analysis was performed with the ^{32}P-labeled genomic *Bam*HI-*Hin*cII fragment (panel C and Fig. 1C) and total RNA from the indicated cell lines. (B) Primer extension analysis was performed with the ^{32}P-labeled 238-bp *Bam*HI-*Xho*I primer (panel C) and total RNA from the cell lines indicated. (C) The S1 probe and primer used in panels A and B are diagrammed. The sizes of the genomic (upper) and cDNA (lower) clones from which they are derived are not drawn to scale.

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                                                    gatccctgcccagctttctctctgcagccc

atatgacccactgcttataagcagtcagaagcccgagcactttccccttcttccaccttg

acttgctgcactctgcactctcactcagacttcactttcctccccttgacgactaaagata

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AACTAATAGATGGAAGATCACAGGAGGATGGGGAGGAGTTGGCAGATCCGCCCCCCAAAA

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TTCAGAGTTCTCACACACAGAAGAATGGACCA/gtaagctgtgcacacctacagacaacc
                                 splice
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FIG. 3. Nucleotide sequence of $I_\gamma 2b$ and the 5′-flanking region. The nucleotide sequence of the region between the *Bam*HI and *Hin*cII sites upstream of $S_\gamma 2b$ (Fig. 1C) was determined. The $I_\gamma 2b$ exon (spliced onto the $C_H 1$ domain of $C_\gamma 2b$) is denoted by uppercase letters. The most-5′ uppercase letter and 3′ (*) transcription initiation sites were determined by the analysis described in the legend to Fig. 2. Stop codons are underlined.

S1 probes prepared from γ3 and γ1 cDNA sequences did not detect transcripts in any of these A-MuLV transformants (data not shown), indicating that the γ2b region is specifically activated in these cells. Germ line γ2b transcripts were also detected in RNA from normal adult spleen cells which also frequently switch to γ2b (Fig. 4A); the latter findings indicate that the expression of germ line γ2b transcripts is not restricted to very early B-lineage stages or to transformed cells. In addition, no germ line γ2b transcripts were detected in L cells (Fig. 4A) or in adult thymus (data not shown), indicating that the production of these transcripts is lineage specific.

Germ line γ2b transcripts were detected in the RNA of four mouse strains (BALB/c, NIH-Swiss, CB-17, and Columbia; Fig. 4), indicating the conservation of structural and regulatory sequences. Preliminary characterizations suggest that similar transcripts were produced from unrearranged C_ε, $C_\gamma 3$, or C_α regions in cells that actively switched to the corresponding C_H genes (J. Stavnezer, G. Radcliffe, and E. Severinson, *in Nobel Symposium 1987*, in press; Rothman, Lutzker, and Alt, manuscript in preparation). Because none of these transcripts appear to encode proteins, potential functional roles associated with their production appear regulatory in nature. A-MuLV transformants switch frequently to γ2b but not to other isotypes (reviewed in reference 14). Constitutive expression of germ line γ2b transcripts in these lines, but not transcripts derived from other germ line C_H regions, correlates with this predisposition. This correlation supports the notion that the expression of germ line C_H transcripts is related to mechanisms that regulate specific class switch events in the context of an accessibility mechanism.

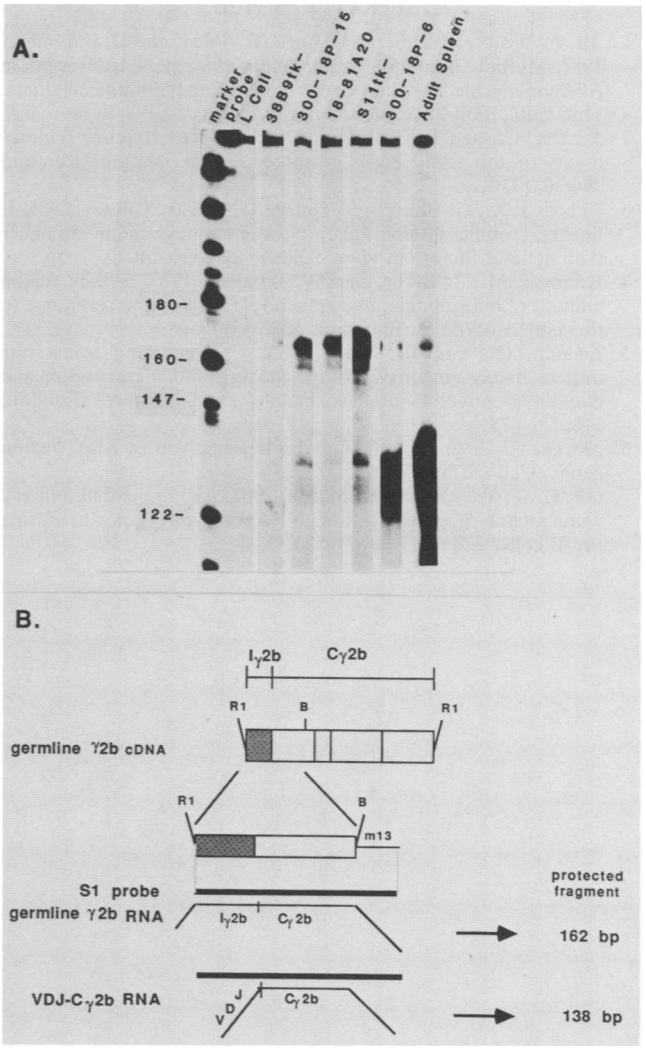

FIG. 4. Expression of germ line γ2b transcripts. (A) Total RNA was prepared from the indicated cell lines and from adult murine spleen (Columbia strain) and assayed for hybridization to a probe specific for germ line γ2b transcripts by the S1 nuclease protection assay outlined in panel B. (B) The 5′ end of germ line γ2b cDNA, from the *Eco*RI site in the linker to the *Bam*HI site in the $C_H 1$ domain of $C_\gamma 2b$, was subcloned into M13 (m13) bacteriophage for use in the S1 nuclease protection assay as diagrammed. Restriction endonuclease sites: R1, *Eco*RI; B, *Bam*HI.

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ADDENDUM IN PROOF

We have recently demonstrated that treatment of B-lineage cells with outside agents such as lipopolysaccharide and interleukin-4 alters the expression of germ line γ2b transcripts and, in turn, modulates the rate of γ2b class switching (S. Lutzker, P. Rothman, R. Pollock, R. Coffman, and F. W. Alt, Cell, in press).

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