Interaction of *H-2Eb* with an IAP retrotransposon in the A20/2J B cell lymphoma

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**Abstract.** In the A20/2J BALB/c B cell lymphoma, Southern analysis revealed an insertion of approximately 6 kilobases of DNA into the first intron of one *Eb*-allele. Two observations suggest that the rearrangement did not occur recently in the A20/2J subline. Firstly, normal and altered *Eb*-alleles are present in equal numbers, and secondly, the LB 27.4 and LS 102.9 somatic cell hybrids formed at an earlier date both possess the rearrangement. Sequences of two cDNA clones, *λEb*-7 and *XEb*-125, selected from an A20/2J cDNA library prepared from poly[A+] RNA indicate that the rearranged *Eb*-allele directs the synthesis of atypical *Eb* transcripts. The clones contain *Eb* sequence linked to a portion of retroviral-like intracisternal A-particle (IAP) genomic sequence, and they appear to be copies of mRNA produced by splicing between a 5′ donor site in the retroviral transcript and the 3′ acceptor site of the *Eb* gene’s first intron. The longer *λEb*-125 insert corresponds to RNA that initiated in the 5′-untranslated region of the *Eb* gene. The 3′-end of the first *Eb* exon joins to long terminal repeat sequence, and retroviral sequence extends up to the splice junction with the second *Eb* exon; 3′ of the junction, the *XEb*-125 sequence corresponds to that of a correctly spliced *Eb* transcript. It seems feasible that the cDNA clones represent hybrid RNA synthesized by read-through transcription of the *Eb* coding region and an IAP element inserted into the first intron of the rearranged *Eb*-allele.

**Introduction**

Intracisternal A particle (IAP) genomes are endogenous retrovirus-like elements, approximately 1 000 copies of which are present in the haploid mouse genome (Hawley et al. 1987). When synthesized in embryonic and transformed cells, the products of IAP genes form particles by budding at the endoplasmic reticulum (Dalton et al. 1961). Unlike retroviral proviruses, IAP have no extracellular phase, and they are therefore not infectious; instead they reside intracellularly, mainly in the cisternae of the endoplasmic reticulum. Several important features are shared with proviruses however, such as an RNA genome coding for IAP structural protein, and an intrinsic reverse transcriptase (Wilson and Kuff 1972). IAP elements are of interest because they appear to participate in transpositions in tumor cells to sites beside or within genes encoding important biological molecules (Hawley et al. 1987). Recorded transpositions are as follows: to the interleukin 3 (IL-3) and Hox-2.4 homeobox loci in the WEIH-3B leukemia line (Ymer et al. 1985; Blatt et al. 1988), to the IL-6 locus in the MPC11 mouse plasmacytoma cell (Blankenstein et al. 1990), to the *c-mos* locus in the XRPC24 plasmacytoma cell (Canaani et al. 1983), and to the κ light chain immunoglobulin locus in two variants of the SP6 hybridoma (Hawley et al. 1984). All of these somatic IAP transpositions alter expression of the mouse gene involved. Gene expression can be activated, as in the myelomonocytic leukemia line WEHI-3B where an IAP genome has inserted 215 base pairs (bp) upstream of the putative IL-3 TATA box; transcription of the IL-3 gene appears to be induced by an IAP long terminal repeat enhancer sequence (Ymer et al. 1985). Alternatively, analysis of the SP6 hybridoma variants revealed that κ light chain transcription was greatly reduced by the insertion of IAP elements into the introns of a κ gene (Hawley et al. 1984). In this report we analyze a somatic rearrangement of the *Eb* gene involving the transposition of an IAP element in the BALB/c A20/2J B cell lymphoma. We observed the rearrangement while searching for lymphoma-specific restriction fragment length polymorphism (RFLP) in major histocompatibility complex (MHC) class II genes by Southern analysis of restriction endonuclease digested nuclear DNA
samples from Balb/c (H-2\textsuperscript{d}) derived A20/2J lymphoma cells. It had previously been reported that A20/2J variant cells that have lost expression of MHC class II gene products arise spontaneously during in vitro culture of the cell line (Tite and Janeway 1981; Jones et al. 1986). Our experiments were originally initiated to determine whether the variations in expression were due to genomic rearrangement. Although rearrangement does not appear to be the cause of the spontaneous loss of MHC class II expression, a rearrangement of the Eb locus does characterize the A20/2J cell line (B. Jones, unpublished observations). In this report we present evidence that the rearrangement appears to be a model for the modification of a MHC class II gene by retroviral transposition.

**Materials and methods**

**Mice and cell lines.** Balb/cByJ mice were from The Jackson Laboratory (Bar Harbor, ME). The A20/2J BALB/c (H-2\textsuperscript{d}) B cell lymphoma, and LB27.4 (\(A^d\), \(E^E\)) and LS102.9 (\(A^d\), \(E^E\)) somatic cell hybrids formed between A20/2J cells and T lymphocyte depleted spleen cells from C57BL/10 and B10.S (7R) mice (Kappler et al. 1982) were obtained from the American Type Culture Collection (Rockville, MD). The cell lines were propagated in vitro as previously described (Jones et al. 1986). Sublines of A20/2J were cloned by limiting dilution using established procedures (Henry et al. 1980).

**Molecular Probes.** The pcl-Eb plasmid (Rogers et al. 1985) is a 500 bp 3' \(Eco\) \(Pst\) fragment at the 5' end of the pGEM-1 (Promega, Madison, WI). It was a generous gift provided to B. Jones and C. A. Janeway Jr. (Yale University, New Haven, CT) by Dr. R. N. Germain (National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD). The pcl-Eb 5' plasmid is a 2.3 kilobase pair (kb) Bam H1-Eco R1 fragment spanning Eb exon 1 (genomic probe-a in Figure 1) inserted into the multiple cloning site of pGEM-4 (Promega, Madison, WI). The probe-a fragment was derived from a clone of a 13.3 kb Eco R1 fragment containing the 5' region of the Eb locus isolated from an Eco R1 partial library of A20/2J nuclear DNA (Scholl et al. 1992). The pcl-Eb 2/3' plasmid is a 475 bp \(Pst\)/Eco fragment containing Eb exon 1 and exon 2 sequences (probe-b in Figure 1) inserted into the multiple cloning site of pGEM-4. The pcl-Eb 2/3' plasmid is a 788 bp \(Pst\)/Eco R1 3' cDNA fragment (probe-c in Figure 1) inserted into the multiple cloning site of pGEM-4; the fragment extends from the \(Pst\) site in Eb exon 2 through exons 3, 4, 5, and 6, and terminates at the Eco R1 site introduced at the 3' end of the cDNA during library construction. Both cDNA probes were derived from the prototypal, full length Eb cDNA clone \(E\beta 2\) isolated from our A20/2J cDNA library in the initial screening with the pcl-Eb cDNA probe (p Eb S8, p Eb 2/5'), and pcl-Eb 2/3' recombinant plasmids are available for distribution.

**Southern analysis.** High molecular mass DNA was purified from BALB/c mouse livers and specified cell lines by established procedures (Maniatis et al. 1982a). After restriction endonuclease digestion, DNA was size-fractionated by agarose gel electrophoresis, treated with acid and alkali, neutralized, and transferred to nitrocellulose filters using established procedures (Maniatis et al. 1982b). Agarose gels (0.8 percent, weight per volume) each of polyvinyl pyrrolidone, ficoll, and bovine serum albumin (BSA), and 100 mg/ml heat denatured, sheared salmon sperm DNA. The filters were hybridized at 42 °C for 16 h. Then they were washed in three high stringency washes of 0.2 x SSC with 0.1 percent SDS, each for 30 min at 55 °C, and exposed using intensifying screens (Cronex lightning plus, Du Pont De Nemours, Wilmington, DE) to X-ray film (X-OMAT; Kodak, Rochester, NY) for 24 h. Before hybridization to a different probe, the filters were stripped by two one hour, 65 °C incubations in 1 liter of 1 mM sodium pyrophosphate.

**cDNA library construction.** The A20/2J cDNA library of about \(1 \times 10^6\) recombinant phage particles was prepared in Lambda from 1 mg poly [A \textsuperscript{+}] RNA using oligo(dT)-primed first strand synthesis and RNase H and DNA polymerase I mediated second strand synthesis (Gubler and Hoffman 1983). Screening at high stringency with nick-translated \(32\text{P}\) probes was performed according to established procedures (Maniatis et al. 1982d).

**DNA sequence analysis.** The DNA sequences of phage clones were determined by double-stranded sequencing of overlapping restriction fragments subcloned into the pGEM-4 plasmid. The dideoxy chain-termination technique (Sanger et al. 1977) was used with sequenase enzyme (USB, Cleveland, OH) and T7 and SP6 sequencing primers (BRL Laboratories, Gaithersburg, MD). Computer assisted sequence analysis was performed using DNASIS software (Hitachi, Yokohama, Japan).

**Results**

One copy of the Eb\textsuperscript{d} allele has undergone somatic rearrangement in the A20/2J cell. We searched for lymphoma-specific RFLP in MHC class II genes by Southern analysis.