Hematopoietic Lineage-Specific Heterogeneity in the 5'-Terminal Region of the Chicken Proto-*myb* Transcript

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Comparison of the nucleotide sequence of the upstream *c-myb* exon UE3 with the sequences of a thymus *c-myb* cDNA and of a B-lymphoma *c-myb* cDNA suggested the existence of T- and B-cell-specific heterogeneity in the 5'-terminal region of the *c-myb* coding sequence. This possibility was investigated with T-cell-specific and B-cell-specific DNA probes in a Northern (RNA) blot analysis of mRNAs from different hematopoietic cell types and from chicken embryo fibroblasts. The hematopoietic tissues analyzed were bone marrow, bursa of Fabricius, and thymus from 1-day-old chicks, 13-day yolk sac, and spleen from 16-day embryos. At least three different *c-myb* mRNA species were found to have 5'-terminal heterogeneity that was specific for either B cells, T cells, or the other hematopoietic cells and chicken embryo fibroblasts. This lineage-specific heterogeneity in the *c-myb* transcript was found to be expressed in the bone marrow precursors of B and T cells before they migrated to their definitive differentiation sites. S1 nuclease protection analysis of the UE3 exon, part of which appeared to be coding sequences for thymic *c-myb* mRNA, revealed that this exon is utilized either in its entirety or partially in a cell-lineage-specific manner by all six tissues analyzed. Also, the 5'-terminal exon(s) present in the thymus cDNA was absent in *c-myb* mRNAs from the other cell types analyzed.

The chicken proto-oncogene *c-myb* is the progenitor, by truncation and transduction, of the retroviral oncogenes (*v-myb*) of avian myeloblastosis virus (AMV) and avian leukemia virus E26 (for a review, see reference 22). Also, the chicken and murine *c-myb* genes are targets for proviral insertions that lead to hematopoietic malignancies of myeloid or B-cell origin (15, 19, 31, 35).

The *c-myb* proto-oncogene, which has been highly conserved throughout evolution, is still largely undefined, but it is large and complex (12, 16, 23, 26). To date, 10 *c-myb* coding exons totaling 1.4 kilobase pairs (kbp) have been identified in chicken chromosomal DNA (14, 36; unpublished data). They represent 69% of the coding sequence but only 38% of the mRNA and are spread over 28 kbp of genomic DNA. The *c-myb* genes in chickens and in other species appear to exist as a single genetic locus, as determined from the absence of restriction-enzyme-fragment-length polymorphism during the screening of different genomic libraries (21, 23, 28, 29). The different human *myb*-related genes that have recently been detected show extensive divergence with each other over most of their nucleotide sequences (25). Chicken *c-myb*-related genes probably exist also but could be easily recognized by the extensive divergence of their nucleotide sequences. In metazoans, the *c-myb* gene encodes a nuclear phosphoprotein of M_r 75,000 to 80,000 that is associated primarily with the nuclear matrix, has a short half-life, binds to DNA, and seems to be a transcription regulator (6, 22, 26).

There is a complex regulation of *c-myb* expression at the transcriptional and posttranscriptional levels that varies in different tissues. The chicken *c-myb* transcript of 4 kbp is detected primarily in immature hematopoietic cells (5, 8, 10, 13, 33, 42) but also in neuronal and connective tissues (38, 39). A decrease in *c-myb* expression correlates with differ-

entiation of lymphoid, myeloid, and neuronal cells (4, 38, 39, 42). In replicating chicken embryo fibroblasts (CEF) and B-lymphoma cells, there is posttranscriptional regulation of *c-myb* expression, with a maximal level during late G1 to early S phase of the cell cycle (39). This regulation does not seem to occur in neuronal cells or in thymocytes (38, 39). There is a block to transcription elongation in the first intron of murine *c-myb* that appears to cause the drastic down-regulation of *c-myb* expression in mature B cells in comparison with pre-B cells in murine B-lymphoid tumor cell lines (4).

Structurally different *c-myb* mRNAs or proteins might be expected from the apparently varied and tissue-specific regulation of *c-myb* expression. For example, alterations in the coding region could affect the function of the gene product, while alterations in noncoding exons could have tissue-specific differential effects on splicing, mRNA stability, or regulation of translation (20, 30, 40). Several mechanisms exist to generate heterogeneous mRNAs from a single gene; these include multiple transcription initiation sites, alternative splicing, 3'-terminal cleavage, differential polyadenylation, and gene rearrangement (7, 20, 30, 34). In murine hematopoietic cells, there is considerable heterogeneity at the 5' terminus of *c-myb* mRNA due to the use of multiple transcription initiation sites (3, 41). Some initiation sites show a quantitative and qualitative preference in different hematopoietic lineages.

The existence of tissue-specific 5'-terminal heterogeneity in chicken *c-myb* mRNA was suggested after comparison of two cDNA clones (11, 32). The nucleotide sequence of a *c-myb* cDNA clone isolated from normal thymus (32) differs completely at its 5' terminus from that of a *c-myb* cDNA clone isolated from B-lymphoma cells (11). Tissue-specific 5'-terminal heterogeneity became more evident during the course of our studies to identify and characterize the 5'-terminal region of the chicken *c-myb* gene. To investigate this phenomenon further, probes specific for the 5'-terminal region of the thymic and the B-lymphoma *c-myb* transcripts

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were prepared for analysis of mRNA isolated from different hematopoietic cells. The results show the existence of at least three species of *c-myb* transcripts, each of which has a 5'-terminal region specific for either thymocytes, B cells, or other hematopoietic cells.

MATERIALS AND METHODS

Cells. The embryonated chicken eggs and newly hatched chicks were White Leghorns obtained from a local supplier (Redwing Hatchery, Los Angeles, Calif.). Yolk sac cells were prepared from 13-day embryonated eggs as described previously (2), and the blood cells were separated by Percoll density gradient sedimentation (5). Spleens were explanted from 16-day embryos; bone marrow, bursas of Fabricius, and thymuses were explanted from 1-day-old chicks. CEF were prepared from 11-day-old embryos and cultured in BM II medium supplemented with 5% (vol/vol) heat-inactivated chicken serum, 5% (vol/vol) calf serum, and 10% (vol/vol) tryptose phosphate broth (2). Exponentially replicating CEF (approximately 5×10^6 cells per 100-mm-diameter culture dish) were used for RNA isolation.

Recombinant *c-myb* clones. The *v-myb*-derived recombinant pBR322 clone HAX-4 and the chicken genomic DNA- λ bacteriophage recombinant clone λ 121, which contains seven *c-myb* exons, were described previously (27, 37). The genomic clone (p640) is a 640-bp *Pst*I fragment containing the *c-myb* upstream exon 3 (UE3) inserted into plasmid pUC18. It was isolated from the 5'-terminal 8.1 kbp *Eco*RI genomic DNA fragment present in clone λ 121 and subcloned into pUC18 (p121-8). The 640-bp *Pst*I fragment was subsequently subcloned into phage M13mp18 for determination of its nucleotide sequence. Derivation of these recombinant clones is summarized in Fig. 1. Thymic *c-myb* cDNA cloned into the *Eco*RI site of pBR322 was kindly provided by Prem Reddy (Wistar Institute).

Restriction endonuclease digestion and gel electrophoresis. Restriction endonucleases (Boehringer Mannheim Biochemicals) were used in the buffers provided by the supplier. Digestions were performed with a 10-fold excess of enzyme. The digested DNAs were electrophoresed in 1% agarose gels containing 0.5 μ g of ethidium bromide per ml.

DNA blot analysis. The DNA digested with restriction enzymes was electrophoresed in a 0.7% agarose gel and transferred to GeneScreen Plus (Dupont, NEN Research Products) and analyzed with probes nick translated with 32 P (specific activity, 10^8 cpm/ μ g). Hybridization was performed as recommended by the GeneScreen Plus supplier.

Isolation of RNA and Northern (RNA) analysis. Total RNA was extracted from freshly explanted tissues and from exponentially replicating CEF by the guanidinium isothiocyanate-cesium chloride method (9). Poly(A)⁺ RNA was isolated by oligo(dT) column chromatography (1). The RNA was electrophoretically separated in 1% agarose-0.66 M formaldehyde gels, transferred to a nitrocellulose filter, irradiated with UV light, and hybridized to DNA probe (10^6 cpm/ml) nick translated with 32 P (specific activity, 10^8 cpm/ μ g). Hybridization was performed in 50% deionized formamide-1 M sodium chloride-0.2% polyvinylpyrrolidone-0.2% bovine serum albumin-0.2% sodium pyrophosphate-1% sodium dodecyl sulfate-10% dextran sulfate-100 μ g of denatured salmon sperm DNA per ml at 42°C for 24 h. The filter was washed by repeating each of the following procedures twice: (i) 5 min at room temperature with $2 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate); (ii) 30 min at 65°C with $2 \times$ SSC-0.5% sodium dodecyl sulfate; and (iii)

FIG. 1. Partial organization of the 5'-terminal region of the chicken *c-myb* gene and derivation of *c-myb* probes specific for B or T cells. (A) Organization of *c-myb* *Eco*RI fragments in the region transduced into AMV. This region contains three 5' exons (□) that were not transduced and seven exons (■) that were transduced within the AMV genome. UE3 is the most 5'-terminal exon in the *c-myb* region shown. Additional exons exist in the downstream and upstream regions not shown. (B) Construction of PSA117 (110 bp) and SAN102 (102 bp), which are *Pst*I-*Sac*II and *Sac*II-*Not*I contiguous genomic subfragments of p640, respectively. BL and T represent the positions at which homology starts with the B-lymphoma and thymic cDNA clones, respectively. ATG is the putative translation initiation codon for the B-lymphoma mRNA and the putative second methionine codon for thymic mRNA. SDS (splice donor site) indicates the 3' end of the exon. (C) Construction of ESA126 (126 bp), which consists of the 5'-terminal 126 bp of the 3.5-kbp thymic *c-myb* cDNA clone. The 5' ATG represents the putative translation initiation codon; the second ATG represents the putative translation initiation codon for B-lymphoma mRNA. AMV indicates the position at which AMV homology starts. Abbreviations: E, *Eco*RI; F, *Fok*I; Ha, *Hae*II; K, *Kpn*I; N, *Not*I; P, *Pst*I; Sa, *Sac*II; X, *Xma*II; Xb, *Xba*I.

30 min at room temperature with $0.1 \times$ SSC. For stringent conditions, the filter was further washed for 30 min at 65°C with $0.1 \times$ SSC. The filter was exposed to Kodak XAR-5 film (Eastman Kodak Co.) with an intensifying screen at -70°C for the time periods stated in the figure legends.

Nucleotide sequence analysis. Sequencing by the dideoxy-chain termination procedure was performed on both strands of selected DNA fragments subcloned into the M13mp18 phage vector as recommended by United States Biochemical Corp.

Probes. The two genomic *c-myb* probes, SAN102 (102 bp) and PSA117 (110 bp), were prepared by digesting plasmid p640 with *Sac*II plus *Not*I and *Pst*I plus *Sac*II, respectively, gel electrophoresis, and electroelution. The thymus-specific probe, ESA126 (126 bp), was prepared by digesting the 3.5-kbp thymic *c-myb* cDNA with *Eco*RI plus *Sac*II and isolating the 5'-terminal 126-bp fragment. The derivation of these probes is shown in Fig. 1.

FIG. 2. Hybridization of mRNA from various tissues with thymus- and B-lymphoma-specific probes. Poly(A)⁺ RNA was prepared from CEF (lane 1), bone marrow (lane 2), yolk sac (lane 3), embryonic spleen (lane 4), bursa (lane 5), and thymus (lane 6). A 10- μ g sample of poly(A)⁺ RNA was used for CEF; 2 μ g was used for the other five tissues. After transfer to nitrocellulose paper, the RNA was hybridized to HAX-4 (A), ESA126 (B), or PSA117 (C). Autoradiographic exposure time was 2 days for panel A and 3 days for panels B and C. The blots were individually prepared, and those in panels B and C were checked for the presence of mRNA by reprobing with the chicken β -actin probe pA1 (17).

S1 nuclease protection analysis. The method used was essentially that described by Maniatis et al. (24). The 640-bp genomic DNA fragment subcloned in pUC18 (p640), which contains the most 5' exon and parts of adjacent presumptive introns, was used as the template after digestion of the p640 clone with *Pst*I, gel electrophoresis, and electroelution. Template DNA (0.1 μ g) and poly(A)⁺ RNA (5 μ g) isolated from each cell type plus yeast tRNA (100 μ g) were hybridized at 60°C. After treatment with S1 nuclease at 20°C, the S1-resistant products were separated by electrophoresis in 2.0% neutral agarose gel and transferred to GeneScreen Plus. The filter was hybridized to the appropriate randomly ³²P-labeled DNA probe (10⁸ cpm/ μ g), washed, and autoradiographed as recommended by Dupont.

RESULTS

Comparison of thymus and B-lymphoma *c-myb* cDNA sequences with *c-myb* genomic sequences. In the 5'-terminal region of the chicken *c-myb* gene (Fig. 1A), the 8.1-kbp *Eco*RI DNA fragment contains a single *c-myb* exon designated UE3 (14, 36; unpublished data). The 8.1-kbp *Eco*RI fragment was inserted into recombinant plasmid p121-8, and UE3 was located in the 3'-terminal 640-bp *Pst*I fragment of p121-8 by hybridization to the 5'-terminal 0.8-kbp *Eco*RI fragment (Fig. 1C) of the thymic cDNA clone of Rosson and Reddy (32) (data not shown). The 640-bp *Pst*I fragment was cloned into plasmid pUC18 (p640) and then into phage M13mp18, and its nucleotide sequence was compared with the published chicken B-lymphoma *c-myb* cDNA sequence of Gerondakis and Bishop (11) and with the sequence of the thymus *c-myb* cDNA (32). As previously reported (14, 36), the B-lymphoma cDNA homology with the genomic DNA extends 21 bp upstream of the point of divergence between the two *c-myb* cDNA sequences, whereas the thymus cDNA homology with genomic DNA ends at the point of divergence. The putative translation initiation codon present at position 63 in the thymic cDNA is absent in the p640 genomic sequence, suggesting the existence of another thymus-specific coding exon that should be located further upstream than the 8.1-kbp *Eco*RI fragment in genomic DNA (Fig. 1A). In the genomic DNA, however, the consensus splice acceptor site sequence (6PyNCAG) that is found at most intron-exon junctions is absent at the point of divergence between the two cDNAs, but such a site is present 17 bp upstream of the start of the B-lymphoma cDNA.

Hybridization of T- and B-cell-specific *myb* probes to mRNA from different hematopoietic tissues. To investigate further the possibility of tissue-specific heterogeneity, T- and B-cell *myb*-specific probes were prepared and hybridized to mRNAs isolated from various hematopoietic cell types and from CEF known to express *c-myb*. Histologically, these six types of tissues represent (i) connective tissue (CEF), (ii) myeloid cells (16-day embryonic spleen), (iii) myeloid and erythroid cells (13-day yolk sac), (iv) B cells in various stages of differentiation (bursa of Fabricius from 1-day-old chick), (v) T cells in various stages of differentiation (thymus from 1-day-old chick), and (vi) immature cells of all hematopoietic lineages (1-day-old chick bone marrow).

The thymus-specific probe (ESA126) consisted of the first 126 bp of the thymic cDNA, in which the *Sac*II site is conveniently located 1 bp after the diverging point of the thymic and B-lymphoma cDNAs. Digestion of the thymic cDNA clone with *Eco*RI and *Sac*II generated a 126-bp fragment with only 5 bp at the 3' end that were also present in the homologous region of the B-lymphoma cDNA (Fig. 1C).

The B-cell-specific probe (PSA117) consisted of the first 110 bp of our genomic clone p640 (Fig. 1B). It was generated from the p640 clone by digestion with the enzymes *Pst*I and *Sac*II. The *Sac*II site is the same as that used to generate the ESA126 probe. Consequently, PSA117 overlaps at the 3' end with 5 bp of the thymic cDNA and with 26 bp of the B-cell lymphoma cDNA.

HAX-4 plasmid DNA, which contains several internal *c-myb* exons transduced into AMV, served as the control to ascertain the expression of any *c-myb* messages.

Poly(A)⁺ RNA from the six tissues described earlier was analyzed by the Northern blot technique with the above-described probes nick translated with ³²P. A relatively larger quantity of poly(A)⁺ mRNA (10 μ g per lane) was needed to detect *c-myb* transcripts in CEF. The five other tissues expressed sufficient *c-myb* RNA to require only 2 μ g per lane. In all six chicken tissues, a relatively uniform size (4.0 kbp) of *c-myb* mRNA was detected with the HAX-4 probe (Fig. 2A). This result is unlike the findings obtained with murine and human tissues, in which *c-myb* RNA species of detectably different sizes have been reported.

When the thymus-specific probe ESA126 was hybridized to mRNA from these tissues, the 4.0-kbp *c-myb* mRNA was detected only in thymus and in bone marrow cells (Fig. 2B).

The hybridization of ESA126 to mRNA from bone marrow cells indicates that hematopoietic cells committed to become T cells express T-cell-specific *c-myb* mRNA before migrating to the thymus.

For hybridization of the B-cell-specific probe PSA117, the filter paper was washed under nonstringent conditions in case the probe contained only the relatively short 26-bp sequence specific for B cells. Only mRNA from bone marrow cells and bursa of Fabricius cells hybridized to the 110-bp genomic sequence present at the 5' end of the p640 insert (Fig. 2C). Thus, like the T-cell precursors, the B-cell precursors expressed their specific *c-myb* transcripts before leaving the bone marrow.

Importantly, the myeloid cells from the embryonic spleen and the myeloid and erythroid cells from the yolk sac as well as the CEF did not hybridize with either the B-cell-specific or the T-cell-specific probe, indicating that they too have their own specific 5'-terminal heterogeneity in *c-myb* mRNA. These results show that there are at least three different *c-myb* mRNA species, each of which is expressed exclusively or predominantly either in thymus, bursa, or other hematopoietic tissues and CEF.

S1 nuclease protection analysis. We next investigated whether that part of *c-myb* UE3 utilized by T cells was also retained by *c-myb* mRNA from CEF, yolk sac cells, or embryonic spleen cells that did not hybridize to the first 21 bp of UE3, i.e., the B-cell-specific probe. The *c-myb* mRNA from B cells contains the entire exon (at least 162 bp), while mRNA from thymocytes contains the last 141 bp (Fig. 1B). For this purpose, a modified S1 nuclease protection analysis was carried out with mRNA isolated from the six *c-myb*-expressing tissues described earlier. The 640-bp genomic DNA insert of plasmid p640, which contains UE3, was used as the template. After hybridization with mRNA and digestion of the products with S1 nuclease, the resistant hybrids were electrophoresed in a 2% neutral agarose gel, transferred to GeneScreen Plus, and hybridized either to the 102-bp *SacII*-*NotI* fragment (SAN102) of clone p640 or to the B-cell-specific probe PSA117. Probe SAN102 contains 102 of the 141 bp in the exon that are common to the thymic and B-lymphoma cDNAs; probe PSA117 contains 21 bp that are unique to B-lymphoma cDNA (Fig. 1B). To prevent the generation of irrelevant fragments of varying length, electrophoresis was carried out in a neutral agarose gel instead of a denaturing polyacrylamide gel because there are several mismatched regions between the two available *c-myb* cDNA clones and the genomic clone.

In addition to detecting the self-reannealed 640-bp DNA template, probe SAN102 detected an S1-resistant fragment of 120 to 160 bp with mRNA from all six tissues (Fig. 3A). (The fragment is present in lane 1 of Fig. 3A but shows poorly in the photograph.) The fragment rendered S1 resistant with mRNA from bone marrow or bursa cells (lanes 2 and 5) appeared to be slightly longer (approximately 160 bp), as expected. However, resolution of the gel was insufficient to allow us to ascertain whether two bands were present in lanes 2 and 5. The B-cell-specific probe PSA117 detected an S1-resistant fragment of approximately 160 bp only with mRNA from bone marrow and bursa cells (Fig. 3B). These results show that UE3 was utilized either in its entirety or partially in a cell-lineage-specific manner by all six tissues analyzed. The internal splice acceptor site utilized in thymocytes is also used either as a splice acceptor site or as a transcription initiation site in CEF, yolk sac cells, embryonic spleen cells, and bone marrow cells, whereas the 5' end of

FIG. 3. S1 nuclease protection analysis. A 20- μ g sample of poly(A)⁺ RNA from CEF (lane 1) or 5 μ g of poly(A)⁺ RNA from bone marrow (lane 2), yolk sac (lane 3), embryonic spleen (lane 4), bursa (lane 5), or thymus (lane 6) was hybridized to the 640-bp genomic DNA fragment (0.1 μ g) from the recombinant plasmid p640. After S1 nuclease digestion, the DNA-DNA and RNA-DNA hybrids were electrophoresed in a 2.0% neutral agarose gel in duplicate, transferred to GeneScreen Plus, and hybridized to probe SAN102 (A) or to the B-lymphoma-specific probe PSA117 (B). Autoradiographic exposure time was 7 days. The length markers (sizes shown on the left) were fragments from phage ϕ X174 DNA digested with *Hae*III; fragments longer than 603 bp are not shown.

the exon is utilized either as a splice acceptor site or as a transcription initiation site by B cells.

DISCUSSION

With a T-cell-specific *c-myb* probe derived from normal thymus cDNA and with a B-cell-specific *c-myb* probe derived from chicken genomic DNA, we have directly demonstrated in *c-myb* mRNA the existence of 5'-terminal heterogeneity specific for T or B cells. Indirectly, i.e., by lack of hybridization to *c-myb* mRNA, these probes also demonstrated the existence of *c-myb* RNA species specific to other hematopoietic cells and to CEF. It remains to be determined whether each of these other cell types (erythroid and myeloid precursors) as well as CEF also possesses its own *c-myb* mRNA species. The heterogeneity resides in the 5'-terminal region either within coding exons or in the adjacent noncoding leader region.

This cell-type-specific heterogeneity is not generated by genomic DNA rearrangement because the nucleotide sequence of the genomic DNA in the region of divergence for the mRNAs is the same in DNA from normal peripheral blood, most probably erythrocytes (pSH1 clone of Hahn et al. [14]), or from leukemic myeloblasts (this study; 36.)

To generate their *c-myb* mRNA, thymocytes and their bone marrow precursors utilize a nonconsensus splice acceptor site within the *c-myb* UE3, part of which appears to be a coding sequence for T cells. The region in the chicken thymic *c-myb* cDNA, located between the first ATG (position 64) and the position where homology starts with B-cell cDNA appears, to be a bona fide coding sequence transcribed from an exon(s) located upstream of the *c-myb* region characterized so far. With our ESA126 probe, representing the first 126 bp of the thymic cDNA, we have established the presence of this T-cell-specific sequence in the *c-myb* mRNA from thymus and bone marrow (Fig. 2B). We do not know why this same 5'-terminal thymic cDNA sequence did not hybridize to *c-myb* mRNA from thymus in the hands of Hahn et al. (14). Soret et al. (36) have reported preliminary results indicating that the genomic DNA se-

quence homologous to the first 62 bp of the thymic cDNA clone is located several kilobase pairs upstream of the 8.1-kbp *EcoRI* genomic fragment. Hahn et al. (14) also showed that the first 120 bp of the thymic cDNA clone hybridized to restriction enzyme fragments of genomic DNA but were not present within 8 kbp of the ATG in UE3. The first ATG codon in the thymic cDNA appears to be functional because it is part of a reasonable Kozak consensus sequence (18), and a TAG codon is located in frame 48 bp upstream.

Bursa pre-B cells and their bone marrow precursors appear to utilize the entire UE3, whose 5' terminus serves either as another splice acceptor site or as a transcription initiation site. A consensus splice acceptor site is absent in genomic DNA at the 5' end of the B-lymphoma cDNA, but such a potential site is located 17 bp upstream. There could have been premature termination of cDNA synthesis, and the two 5' terminal bases (CA) nonhomologous with genomic DNA might have been a cloning artifact. On the other hand, the G+C-rich genomic region (124 bp) immediately upstream of the B-lymphoma cDNA is highly conserved (94% homology between chicken and mouse sequences) and contains several canonical binding sites for transcription factors (14, 41; unpublished data). Whether alternative splice sites or different transcription initiation sites exist cannot be determined until the promoter region of the chicken *c-myb* gene is identified or until full-length cDNA copies of the mRNA are obtained. However, because of the apparently uniform size (4 kbp) of the *c-myb* mRNA in all cell types analyzed, the length of the mRNA must be maintained constant by a compensating splicing mechanism. In murine *c-myb* transcripts, which have a heterogeneous size of 3.8 to 4.2 kbp, different transcription initiation sites appear to exist, although the data could also be compatible with the presence of alternative splice sites at the 5' end (3, 41). Alternative splicing further downstream between exons 6 and 7 of the murine *c-myb* transcript has been observed in normal thymus and spleen cells (34) as well as in myeloid tumor cells induced by proviral integration within the 5'-terminal region of the *c-myb* gene (31).

CEF and hematopoietic cells other than B and T cells do not utilize the same 5' terminal exon(s) as do T cells (Fig. 2B) but utilize the same fraction of UE3 as do T cells (Fig. 3A). Again, it is not possible to determine whether *c-myb* mRNAs in these cells utilize the internal UE3 site as a splice acceptor for an additional exon(s) located upstream or whether *c-myb* mRNA synthesis starts there.

The identification of 5'-terminal *c-myb* mRNA sequences that are specific for different hematopoietic cell types may permit us to identify and isolate cell-specific factors that bind to these sequences or, if such sequences are present in the coding region, to identify the target molecules with which they interact.

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