Erythroid-Specific Processing of Human β Spectrin I Pre-mRNA

By Zhi-Liang Chu, Amittha Wickrema, Sanford B. Krantz, and John C. Winkelmann

Erythroid cells express a unique form of β spectrin I as a result of tissue-specific alternative pre-mRNA processing. Nonerythroid cells that express the β spectrin I gene include four additional exons at the 3’ end of the mature transcript, leading to elongation of the carboxyl terminus of the protein. The nonerythroid β spectrin I isoform is not present in the red blood cell membrane skeleton; the erythroid isoform is not detected in other cell types. Therefore, developing erythroid cells acquire this tissue-specific pre-mRNA processing activity during differentiation. In the present study, we investigated the developmental timing of erythroid-specific pre-mRNA processing in human erythroid precursors. Partially purified human peripheral blood blast forming unit-erythroid (BFU-E) cells were grown in culture for 5 to 12 days. β Spectrin I mRNA transcripts were analyzed at different time points by S1 nuclease mapping. The processing of β spectrin I transcripts was found to be exclusively erythroid from day 5 onward, indicating that erythroid-specific processing is not linked temporally to assembly of the mature erythroid membrane skeleton. Human erythroleukemia (HEL) cells had both erythroid and nonerythroid transcripts, indicating that both processing patterns can coexist.

Spectrin is the most abundant protein in the mammalian red blood cell membrane skeleton. Spectrin is a heterodимер, composed of nonidentical α and β subunits. Interestingly, the expression of erythroid α spectrin (α spectrin I) is limited to erythrocytes. Instead, nonerythroid cells express α spectrin II (α fodrin), the product of a homologous but distinct gene. In contrast, erythroid β spectrin (β spectrin I) is expressed in nonerythroid tissues including muscle, heart, and brain. It is often coexpressed with β spectrin II (β fodrin), another distinct but related gene. The structure of the nonerythroid isoform of β spectrin I (β spectrin Σ2, for “subtype 2”) is different than that of erythrocytes (β spectrin Σ1). The mechanism responsible for the structural difference is alternative pre-mRNA processing of β spectrin I transcripts. In erythroid cells, the pre-mRNA transcript is cleaved and polyadenylated after exon 32 (Fig 1). Within exon 32 of the β spectrin I gene, there is a donor splice site that is used in nonerythroid cells to splice in four additional exons, termed 1m-4m for their original identification in muscle (Fig 1). These two processing pathways are mutually exclusive.

The donor splice site in exon 32 is 5’ to the translation termination codon, so that the nonerythroid transcript encodes a different carboxyl terminus (Fig 1). The functional consequence of the different carboxyl terminal structure of β spectrin Σ1 and β spectrin Σ2 is unknown. The nonerythroid carboxyl terminus includes a “pleckstrin homology” (PH) domain that is shared with pleckstrin (the major protein kinase C substrate of platelets), β spectrin II, dynamin, and several molecules involved in signal transduction such as ras-GAP, ras-GRF, Son of sevenless, rac protein kinases, β adrenergic receptor kinase, and others. It has been suggested that PH domains bind to βγ subunits of trimeric G proteins. The impact that this functional attribute might have on β spectrin I function is unknown.

Interestingly, the erythroid β spectrin I pre-mRNA processing pathway is tissue-specific. Erythrocytes have no β spectrin Σ2, and nonerythroid cells do not express β spectrin Σ1. We infer that nonerythroid β spectrin I pre-mRNA processing represents the constitutive pathway. In fact, when cells that normally do not express β spectrin I (eg, HeLa cells) are transfected with a β spectrin I minigene, only nonerythroid processing is evident. Therefore, differentiating erythroid cells acquire the ability to specifically process β spectrin I pre-mRNA, producing the unique erythroid β spectrin Σ1 protein. Presumably, erythroid cells did not evolve this novel pre-mRNA processing machinery for β spectrin I only. There are many examples of erythroid genes that undergo alternative pre-mRNA processing.

In the current study, we examine the timing of the acquisition of erythroid differentiation in HEL cells using hemin resulting in a partial switch toward the erythroid processing pattern of β spectrin I transcripts. Using a genomic S1 probe that spans the erythroid polyadenylation signal, we found that a substantial portion of the transcripts detected by the erythroid cDNA S1 probe (in both cultured BFU-E and HEL cells) is incompletely processed pre-mRNA precursors. Poly(A) RNA selection before S1 analysis showed that the unprocessed transcripts are not polyadenylated. We conclude that (1) erythroid-specific pre-mRNA processing activity is present early in erythroid differentiation; (2) β spectrin I transcripts that are unprocessed at the 3’ and accumulate, awaiting either erythroid or nonerythroid processing pathways, from which observation we infer that the regulated alternative pathways are both inefficient; and (3) HEL cells offer a human cell culture model in which to study the balance between the two pre-mRNA processing pathways. We speculate that erythroid cells evolved this tissue-specific pre-mRNA processing machinery for other erythroid genes in addition to β spectrin I.

© 1994 by The American Society of Hematology.

From www.bloodjournal.org by guest on October 30, 2017. For personal use only.
tion of erythroid-specific pre-mRNA processing activity by developing red blood cells. We use S1 nuclease mapping to examine the structure of β spectrin I transcripts in RNA isolated from cultured human peripheral blood (PB) burst-forming unit-erythroid (BFU-E) cells. Also, we examine the processing of β spectrin I transcripts in human erythroblasts (HEL) cells, a transformed cell line thought to represent a very primitive erythroid precursor. Induction of HEL cell differentiation is examined for its effect on erythroid pre-mRNA processing.

MATERIALS AND METHODS

Materials. The HEL cell line was obtained from the American Type Culture Collection (ATCC; Rockville, MD). Cell culture media, reagents, and sera were purchased from Gibco BRL (Gaithersburg, MD). Human recombinant Epo (rEpo) was a gift from Ortho Pharmaceutical (Raritan, NJ). Reagents for polymerase chain reaction (PCR) were from Perkin Elmer-Cetus (Norwalk, CT). PCR primers were synthesized in the Microchemical Facility of the Institute of Human Genetics (University of Minnesota, Minneapolis, MN). They are JW5: 5'-GAAGCTCTACAAAGGAGCT-3', JW6: 5'-GGACAGCTATAGCCTAAG-3', JW9: 5'-ATATGCGGC-CGCCAGCGTATACAAGATGAC-3', and JW11: 5'-GACATA-GCTTCAAGAGGAGAGCCGA-3'. Radioactive nucleotides (nts) were purchased from Amersham (Arlington Heights, IL). Plasmid pBSII-8BX6470 is a 476-nt XhoI to SacII subclone of the previously described erythroid β spectrin I genomic DNA sequence (172 bp) in plasmid SK II (pBSII) from Stratagene (La Jolla, CA). Plasmid pBSII-8BX6470 is a 476-nt XhoI to SacII subclone of the previously described muscle β spectrin I genomic DNA sequence (172 bp), also in pBSII. These subclones contain DNA segments of identical sequence from the XhoI site to the alternative splice site (304 bp) plus erythroid-specific 3' cDNA sequence (326 bp) in pBSII-8BX6470 and muscle-specific 3' sequence (172 bp) in pBSII-8BX6470. Plasmid pBSII-SCN-1 is a β spectrin genomic DNA subclone containing exon 32 plus 30 bp of DNA 3' to the erythroid-specific cleavage/polyadenylation site (Fig 1). pBSII-SCN-1 was obtained by PCR amplification of β spectrin I genomic DNA using primers JW9 (a NarI recognition sequence and genomic sequence 3' to the erythroid cleavage/polyadenylation site) and JW11 (a HindIII recognition sequence followed by the first 20 nts of exon 32). The amplified fragment was ligated into pBSII.

Cell culture. PB was collected from normal adult donors after informed consent approved by the Vanderbilt University and Veterans Affairs Medical Center review boards. Purification and culture of BFU-E were performed as previously described.19 In brief, light density mononuclear cells were separated from PB by centrifugation over Ficoll-Hypaque (1.077 g/mL), washed twice with α-minimum essential medium (α-MEM), resuspended in 50% α-MEM and 50% Iscove’s modified Dulbecco’s medium (IMDM). These cells were then incubated with sheep erythrocyte cells, and nonrosetted cells were separated from rosette-forming cells by centrifugation over Ficoll-Hypaque. Cells were washed twice with α-MEM followed by overnight incubation in polystyrene flasks at 37°C in a 5% CO2 incubator with 20% fetal calf serum (FCS) and 10% giant cell tumor-conditioned medium (GIBCO, Grand Island, NY) to deplete monocytes. Granulocytes, monocytes, colony-forming unit (CFU)-granulocyte-macrophage, T and B lymphocytes and natural killer cells were removed from the nonadherent cells by coating these cells with a mixture of four monoclonal antibodies: CD11b/OKM1 plus CD2/OKT11 and CD45R/MY11 plus CD16/MY23 and subsequent incubation in polystyrene dishes that have been coated with affinity-purified goat antimurine IgG (Boehringer Mannheim Biochemicals, Indianapolis, IN). The nonadherent cells were cultured at a concentration of 2 x 10^5 cells/mL in 30% FCS, 10% bovine serum albumin, 2 U/mL Epo, 50 U/mL interleukin-3, 10 µg/mL insulin, 5 x 10^-5 mol/L β-mercaptoethanol, 500 U/mL penicillin, 40 µg/mL streptomycin, and 0.9% methylcellulose. Cells collected after 5 to 8 days in culture were further purified and cultured as described.19 The liquid culture medium contained 15% FCS, 15% human AB serum, 1% human serum albumin, IMDM, 1 U/mL rEpo, 10 U/mL insulin, 500 U/mL penicillin, and 40 µg/mL streptomycin. The cultured cells undergo differentiation and mature into reticulocytes at day 14 of culture (day 0 being the day blood was collected from the donors). Therefore, day 7 approximates the CFU-erythroid (CFU-E) stage of development.

HEL cells were maintained in culture using media and conditions recommended by the ATCC. Hemin induction increases the proportion of cells expressing hemoglobin 10-fold (to 80% of total cells) compared with that for spontaneous differentiation. Hemin induction of HEL in this study was performed essentially as described by...
A  ERYTHROID S1 PROBE AND PROTECTED FRAGMENTS

\[ \text{pBSII-\beta 28 XR 623} \]

\[ \text{T7} \hspace{1cm} \text{JW5} \hspace{1cm} \text{R} \hspace{1cm} \text{T3} \hspace{1cm} \text{JW6} \]

POSITIVE CONTROL RNA

gDNA PROBE BY PCR

ERYTHROID

\[ \hspace{1cm} 671 \text{bp} \]

\[ \hspace{1cm} 544 \text{nt} \]

\[ \hspace{1cm} 211 \text{nt} \]

NONERYTHROID

\[ \hspace{1cm} 461 \text{bp} \]

\[ \hspace{1cm} 211 \text{nt} \]

\[ \hspace{1cm} 383 \text{nt} \]

B

1 2 3 4 5 6 7

C

1 2 3 4 5 6 7 8

\[ \leftarrow 461 \]

\[ \leftarrow 383 \]

\[ \leftarrow 211 \]
Martin and Papayannopoulou.20 Actively proliferating cells, with a density of 1 to 2 × 10^5 cells/mL of culture medium (10% fetal bovine serum, 90% RPMI 1640, 200 U/mL penicillin, 200 μg/mL streptomycin), were exposed to 50 μmol/L bemin (from a 2 mmol/L L [40×] stock solution in 10 mmol/L NaOH) for 4 days at 37°C with humidified air and 5% CO2.

RNA isolation. Total RNA was isolated at various stages of erythroid cell culture by hot phenol extraction.21 Briefly, cells were washed in IMDM, and the cell pellets were resuspended in a solution of 10 mmol/L NaAc (pH 5.2)/0.5% sodium dodecyl sulfate. Immediately after resuspension, an equal volume of phenol that had been equilibrated with 10 mmol/L NaAc (pH 5.2) was added and extracted for 5 minutes at 56°C. The solution was rapidly cooled to 4°C in an ice bath followed by centrifugation for 5 minutes at 12,000g. The aqueous phase was extracted a second time, and the RNA was precipitated twice with 0.3 mol/L NaAc (pH 6.3) plus 2.5 vol of ethanol.

Total cellular RNA of cultured HEL cells was isolated using RNAzol according to the procedures described by the supplier (Cinna/Biotec, Friendswood, TX).22 Polyadenylated RNA was fractionated from HEL total cellular RNA by oligo(dT) cellulose affinity chromatography.23

S1 nuclease protection assay. The S1 nuclease protection assay method used in this study is a modification of the method described by Berk and Sharp.24,25 Various uniformly labeled, defined-length, double-stranded DNA probes were synthesized by PCR. RNA samples to be studied were co-precipitated with the double-stranded DNA probe (5 × 10^5 counts per minute [cpm] per hybridization) in 70% ethanol and 0.3 mol/L sodium acetate. After washing in 70% ethanol, the RNA/DNA pellet was resuspended in 30 μL hybridization buffer (40 mmol/L PIPES [pH 6.4], 1 mmol/L EDTA [pH 8.0], 0.4 mol/L NaCl, 80% formamide) and overlaid with mineral oil in a microcentrifuge tube. The hybridization was performed in a PTC-100 programmable thermal controller (MJ Research, Inc., Watertown, MA) with the following sequence: 94°C for 20 minutes to denature the probe and RNA secondary structure with cooling to the predetermined hybridization temperature (see Fig legends) for 12 to 16 hours. In most cases, probe reannealing was minimal at the optimized hybridization temperature. After hybridization, 300 μL ice-cold S1 nuclease solution (0.28 mol/L NaCl, 0.05 mol/L sodium acetate [pH 4.5], 4.5 mmol/L ZnSO4, 20 μg/mL single-stranded carrier DNA, 150 to 200 U/mL S1 nuclease) was rapidly added. S1 nuclease digestion was performed for 90 minutes at 45°C and stopped by adding 80 μL of the stop mixture (4 mol/L ammonium acetate, 50 mmol/L EDTA [pH 8.0], 50 μg/mL yeast tRNA). The protected fragments were extracted once with phenol/chloroform (1:1), precipitated in ethanol, and fractionated on a 4% polyacrylamide/7.6 mol/L urea gel. Autoradiography was used to image the signals.

To examine the point at which developing human erythroid cells acquire tissue-specific β spectrin I transcripts, double-stranded, internally labeled, defined-length DNA probes were synthesized using PCR from the plasmids shown (described in Materials and Methods). The same plasmids were used to synthesize RNA controls, using T7 RNA polymerase, that correspond to erythroid and nonerythroid processing patterns. The probes were synthesized using one primer in the plasmid to facilitate recognition of probe self-anneling (larger protected fragment) as opposed to probe annealing to RNA (smaller protected fragment).

To examine the point at which developing human erythroid cells acquire tissue-specific β spectrin I transcripts, double-stranded, internally labeled, defined-length DNA probes were synthesized using PCR from the plasmids shown (described in Materials and Methods). The same plasmids were used to synthesize RNA controls, using T7 RNA polymerase, that correspond to erythroid and nonerythroid processing patterns. The probes were synthesized using one primer in the plasmid to facilitate recognition of probe self-anneling (larger protected fragment) as opposed to probe annealing to RNA (smaller protected fragment).

RESULTS

Figure 2A shows schematically the probes used to distinguish erythroid from nonerythroid pre-mRNA processing patterns in human β spectrin I transcripts. Double-stranded, internally labeled, defined-length DNA probes were synthesized using PCR from the plasmids shown (described in Materials and Methods). The same plasmids were used to synthesize RNA controls, using T7 RNA polymerase, that correspond to erythroid and nonerythroid processing patterns. The probes were synthesized using one primer in the plasmid to facilitate recognition of probe self-anneling (larger protected fragment) as opposed to probe annealing to RNA (smaller protected fragment).

To examine the point at which developing human erythroid cells acquire tissue-specific β spectrin I transcripts, double-stranded, internally labeled, defined-length DNA probes were synthesized using PCR from the plasmids shown (described in Materials and Methods). The same plasmids were used to synthesize RNA controls, using T7 RNA polymerase, that correspond to erythroid and nonerythroid processing patterns. The probes were synthesized using one primer in the plasmid to facilitate recognition of probe self-anneling (larger protected fragment) as opposed to probe annealing to RNA (smaller protected fragment).
all the transcripts are processed in the erythroid pattern (Fig 2C, erythroid RNA control in lane 8). If day-5 cells (the earliest time point we could examine) are studied or if the culture is extended to 12 days, there are still no detectable nonerythroid transcripts (data not shown). The presence or absence of stem cell factor in the cell culture medium did not influence the processing of β spectrin I pre-mRNA in cultured erythroid cells (data not shown).

To examine a cell representing an earlier stage of erythroid development, HEL cells were studied. HEL cells were established from a patient with erythroleukemia. These cells are capable of differentiation along either erythroid or megakaryocytic lineages, suggesting that they perhaps represent a transformed bipotential erythroid progenitor. Interestingly, these cells have both erythroid and nonerythroid β spectrin I transcripts by S1 analysis (Fig 2C, lane 2), although the erythroid pattern predominates. These two pre-mRNA processing patterns have not previously been observed together in a single cell type.

We studied the effect of erythroid differentiation of HEL cells by hemin on β spectrin I pre-mRNA processing (Fig 3). In this typical experiment, before hemin induction, the ratio of erythroid to nonerythroid transcripts (using densitometric analysis of autoradiographs) is 1.45. After induction, the ratio increases to 2.51, a 73% increase. The same result is obtained whether the erythroid or nonerythroid S1 probe is used and has been reproduced multiple times (data not shown). Therefore, erythroid differentiation of HEL cells results in a partial shift in the relative abundance of erythroid and nonerythroid β spectrin I transcripts. Induction of megakaryocytic differentiation in HEL cells using phorbol esters does not affect alternative processing of β spectrin I pre-mRNA.

The S1 protection probe synthesized from erythroid β spectrin I cDNA will detect splicing of exons 30, 31, and 32 (see Fig 1) as well as the utilization of the nonerythroid donor splice site in exon 32. However, erythroid-specific polyadenylation is not directly measured by this probe because it is synthesized from cDNA that is itself cleaved and polyadenylated at the erythroid site. To directly measure erythroid-specific cleavage/polyadenylation, we synthesized an S1 probe from a plasmid containing a genomic DNA subclone (pBSII-SCNI, described in Materials and Methods). This probe spans the erythroid poly(A) addition site (Fig 4A). Therefore, cleavage/polyadenylation will result in a smaller protected fragment than uncleaved pre-mRNA. Nonerythroid transcripts that use the donor splice site in exon 32 will protect only a very small fragment of this S1 probe, too small to be visualized in autoradiographs of 4% acrylamide gels.

β Spectrin I transcripts in HEL cells protected two fragments of the pBSII-SCNI genomic poly(A) addition site probe (Fig 4B), 402 nts, corresponding to erythroid-specific cleavage and 432 nts, corresponding to the uncleaved precursor pre-mRNA. Poly(A) selection greatly diminished the quantity of the 432-nt species, suggesting that this precursor transcript is not polyadenylated. In this experiment RNA was passed a single time over oligo(dT) cellulose. There was considerable unpolyadenylated RNA remaining in the sample, determined by the persistence of ribosomal RNA. The 432-nt protected fragment in lane 3 of Fig 4B is, we feel, because of incomplete poly(A) selection.

To determine whether this unexpectedly abundant unpolyadenylated transcript is a feature only of HEL cells, we analyzed RNA from cultured erythroid progenitor cells (Fig 4C). Human BFU-E, grown in culture for 6, 8, or 10 days, showed the incompletely processed β spectrin pre-mRNA as well as transcripts that are polyadenylated at the erythroid-specific site. Additional low molecular weight protected...
probe fragments are observed in this experiment that may represent S1 overdigestion or alternative cleavage sites.

We conclude that the 432 nt protected probe fragment detected in the experiments shown in Fig 4 is incompletely processed rather than unprocessed pre-mRNA. If such an abundant species was totally unprocessed, the cDNA probes used in Figs 2 and 3 would have resulted in lower molecular weight fragments corresponding to unspliced exons 30, 31, and 32. Such bands are not observed; therefore, the transcript is spliced but not cleaved or polyadenylated.

**DISCUSSION**

The developmental expression of erythroid-specific genes depends on both the induction of gene transcription and the posttranscriptional regulation of pre-mRNA processing. Transcriptional control of erythroid genes has received considerable attention. In contrast, little is known of the molecular mechanism of erythroid-specific pre-mRNA processing. In this study, we examine the erythroid-specific processing of human \( \beta \) spectrin I pre-mRNA. In addition to spectrin, there are several other examples of proteins that show such regulation. Therefore, a better understanding of the timing and mechanism of regulated \( \beta \) spectrin I pre-mRNA processing might lead to insights of broad significance for erythroid-specific gene expression and erythroid differentiation.

We found that the earliest human erythroid progenitor we could study, PB BFU-E grown in culture for 5 days, already transcribes the \( \beta \) spectrin I gene and processes its pre-mRNA in a completely erythroid manner. In this culture system, day 7 is thought to represent the CFU-E stage of development. Therefore, the erythroid-specific processing activity is present long before \( \beta \) spectrin \( \Sigma 1 \) is needed for assembly of the membrane skeleton. This observation supports the hypothe-
sis that the erythroid-specific processing machinery is not solely for β spectrin I transcripts. We speculate that erythroid-specific pre-mRNA processing activity might be an early marker for erythroid differentiation and may even play a regulatory role in erythropoiesis.

To further examine β spectrin I pre-mRNA processing in erythroid progenitors, we studied the HEL cell line. HEL cells are thought to represent the transformed state of an early erythroid cell. Northern analysis has shown that there are β spectrin I transcripts in HEL cells. Our current data show that β spectrin I mRNA abundance in HEL cells is considerably less than that observed in cultured BFU-E. Our results further show that both erythroid and nonerythroid β spectrin I transcripts are present in HEL cells. Induction of erythroid differentiation using hemin results in a partial shift in the relative abundance of β spectrin I transcripts, with increased erythroid-processed mRNA compared with that for the nonerythroid pattern. We conclude that HEL cells represent a transitional stage of erythroid development. These cells offer a model in which to investigate the molecular basis of erythroid-specific processing and the balance between the competing processing pathways.

We were concerned that the S1 mapping experiments using probes derived from erythroid β spectrin I cDNA did not directly measure erythroid-specific cleavage/polyadenylation. Conceivably, transcripts might exist that have had exons 30, 31, and 32 spliced together (this splicing is detected using the erythroid cDNA probe) but have been neither polyadenylated at the erythroid site nor spliced to exons 1m-4m. Therefore, we designed a probe to directly measure erythroid cleavage/polyadenylation. To our surprise, we found such partially processed transcripts in abundance in both cultured BFU-E and HEL cells. Poly(A) selection showed that these incompletely processed transcripts are not polyadenylated at another site. This observation implies that 3′ processing of β spectrin I transcripts is inefficient in both the erythroid and nonerythroid pathways, at least in HEL cells. Therefore, we speculate that incompletely processed transcripts accumulate, awaiting processing by one or the other pathway. Putative regulatory mechanisms would act to shift the balance between the two processing pathways.

The sequences of the erythroid polyadenylation site of exon 32 and the acceptor splice site of exon 1m are atypical, suggesting a basis for the presumed inefficiency of processing by either route. The AATAAAA signal is 45 bp from the cleavage/polyadenylation site, considerably farther than usual. The site itself (ACACTA*gcgcagagattgattagca, where * denotes the cleavage location) is unusual in that the sequence 3′ to the point of cleavage is not as rich in G and T as is commonly observed. The sequence of the 3′ splice acceptor site on exon 1m is ccctgagcag*GGGGAAG, where the * indicates the exon boundary. The presence of a second AG dinucleotide within 15 bp of the acceptor AG is very atypical. These sites (or others that appear normal) may be recognized by sequence-specific regulatory molecules that affect processing and govern their specificity and efficiency. Alternatively, these atypical sites might reduce processing efficiency enough to allow a more global alteration of the processing machinery to act on the balance between the two competing pathways. An analogous mechanism appears to be operating in the regulation of secreted versus membrane bound Ig µ heavy chain in developing B lymphocytes.

Figure 5 summarizes the various 3′ β spectrin I pre-mRNA processing patterns. Skeletal muscle cells represent the prototype nonerythroid tissue. Because several other nonerythroid cell types (including cardiac muscle and brain) show the same processing, we infer that this pattern is the default, constitutive pathway for β spectrin I processing. As erythroid differentiation occurs, there may be an intermediate stage, represented by HEL cells, where both pathways...
coexist. By the CFU-E stage of development, erythroid-specific processing dominates. Tissue-specific regulatory mechanisms that could act to shift \( \beta \) spectrin pre-mRNA processing into the erythroid pathway are shown in Fig 5. These possibilities are (1) erythroid-specific upregulation of cleavage/polyadenylation at the exon 32 site (indicated by ‘+’ in Fig 5), (2) downregulation of nonerythroid splice sites in exons 32 and 1m (indicated by ‘-’ in Fig 5), and (3) tissue-specific inhibition of transcription between exon 32 and 1m (indicated by ‘x’ in Fig 5). Other potential mechanisms not shown in Fig 5 include differential stability of erythroid and nonerythroid \( \beta \) spectrin transcripts and some combination of the various possibilities. Further studies to delineate the control mechanism will be facilitated by the HEL cell model described in this paper. A better understanding of this phenomenon may lead to fundamental knowledge concerning posttranscriptional control of erythroid gene expression and erythroid differentiation.

ACKNOWLEDGMENT

The authors wish to thank Peggy Garner Hamrick and Jon Vanderwelde for their excellent technical assistance.

REFERENCES

9. Chu Z-L, Winkelmann JC: Regulation of erythroid-specific processing of human \( \beta \) spectrin pre-mRNA may occur at the level of differential polyadenylation. Blood 82:175a, 1993(abstr, suppl)
26. Peterson ML, Gimmi ER, Perry RP: The developmentally regulated shift from membrane to secreted \( \mu \) mRNA production is accompanied by an increase in cleavage-polyadenylation efficiency but no measurable change in splicing efficiency. Mol Cell Biol 11:2324, 1991
27. Peterson ML: Balanced efficiencies of splicing and cleavage-polyadenylation are required for \( \mu \) and \( \mu \) mRNA regulation. Gene Expression 2:319, 1992
Erythroid-specific processing of human beta spectrin I pre-mRNA

ZL Chu, A Wickrema, SB Krantz and JC Winkelmann