Changing patterns in cytoskeletal mRNA expression and protein synthesis during murine erythropoiesis *in vivo*

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ABSTRACT The major cytoskeletal proteins α -spectrin, β -spectrin, and ankyrin are synthesized and assembled into a supportive membrane skeleton during erythroid differentiation. Information on the temporal appearance of mRNA and protein species is essential for understanding both the cytoskeletal assembly process and the function of various isoforms. We have isolated highly enriched populations of fetal erythroid cells at various stages of maturation. mRNAs for erythroid ankyrin, α -spectrin, and β -spectrin were expressed at all stages but there were differences in transcript types and levels. The ratio of 9-kilobase (kb) to 7.5-kb erythroid ankyrin transcripts decreased markedly during differentiation, but there was no change in the ratio of the 10.1-kb and 9.3-kb erythroid β -spectrin transcripts. The relative amounts of ankyrin, α -spectrin, and β -spectrin mRNA increased during yolk sac cell differentiation, whereas only α -spectrin mRNA increased during differentiation of the fetal liver cells. The amounts of β -spectrin mRNA exceeded the amounts of α -spectrin mRNA in the early precursors from both yolk sac and fetal liver; protein synthetic levels showed the same pattern. The 16-day fetal peripheral reticulocytes, on the other hand, had the adult mRNA and protein synthetic ratios with $\alpha/\beta > 1$. The data indicate that at least two mechanisms exist to meet changing erythroid membrane cytoskeletal requirements during development *in utero*: (i) stage-specific processing of the mRNA for the major cytoskeletal linker protein ankyrin and (ii) developmentally regulated α/β -spectrin protein synthetic rates.

The erythrocyte membrane skeleton is composed primarily of tetramers of α (240 kDa) and β (220 kDa) spectrin joined together by junctional complexes consisting of short actin filaments and protein 4.1 to form a two-dimensional network (1, 2). Adducin, protein 4.9, myosin, and tropomyosin are also present at the junctional complexes (1, 2). The spectrin lattice is attached to the erythrocyte plasma membrane through at least two binding sites. One of these is at the junctional complexes and is mediated by protein 4.1–glycophorin linkages (3, 4). The major plasma membrane attachment site is provided by ankyrin (protein 2.1), which contains high-affinity binding sites for both β -spectrin and the transmembrane anion exchanger (protein 3) (5). Binding of the membrane protein 4.2 to the ankyrin complex is thought to stabilize the interaction (6, 7). The integrity of the membrane cytoskeleton is essential to the survival of the red blood cell, since heritable defects in spectrin and/or ankyrin result in severe hemolytic anemia in humans and mice (8–13).

Numerous studies have investigated the synthesis and assembly of cytoskeletal proteins into the red-cell membrane skeleton (14–25). As it is difficult to obtain enriched populations of stage-specific cells *in vivo* from adult animals, most studies have been limited primarily to virus-transformed cell

lines (murine erythroleukemia) or spleen cells induced to differentiate with dimethyl sulfoxide or erythropoietin, respectively (14–20, 22–24). Although the results have been informative, there are inconsistencies among the published observations, especially related to the α/β -spectrin protein synthetic ratios (17, 18, 20, 22). Furthermore, studies of the α/β synthetic ratio by *in vitro* labeling of freshly isolated chicken embryonic cells (26) and phenylhydrazine-treated rat (21) and mouse (27) cells are in agreement with one another but the ratios are different from those noted in the majority of the transformed cells.

As an alternative to studying mixed populations from adult mice or cell lines, we have utilized fetal mouse cells. Murine erythroid differentiation *in utero* occurs in two waves: (i) nucleated yolk sac-derived cells progress from pre-type 1 to type 3 erythroblasts during days 10–14 and (ii) fetal liver cells, primarily pro- and basophilic erythroblasts at 12–14 days, generate predominantly reticulocytes in the peripheral blood by 16 days (28). The transcripts and protein products from erythroid α and β spectrins were investigated simultaneously. Stage-related shifts in mRNA levels and in α/β -spectrin protein synthetic ratios did occur during normal erythroid maturation *in utero*. We suggest that the differences reflect changing requirements of the erythrocyte membrane during the extensive remodeling that accompanies erythrocyte differentiation.

MATERIALS AND METHODS

Animals. Erythroid cells were obtained from normal fetuses by timed matings of C57BL/6J (B6)-+/+ breeding pairs. The appearance of a vaginal plug was designated day 0 of pregnancy. Adult spleen cells and reticulocytes were obtained from B6-+/+ mice treated with phenylhydrazine (P7126; Sigma) as described (29). Phenylhydrazine treatment resulted in pronounced reticulocytosis ($\geq 95\%$) and splenic erythroid hyperplasia.

Isolation of Fetal Cells. Females were killed by cervical dislocation on day 10, 12, 13, 14, or 16 of pregnancy. Fetuses were removed and placed in sterile phosphate-buffered saline (PBS, pH 7.4; 310-4200AG; GIBCO). Fetal livers were dissected in fresh cold (4°C) sterile PBS and dissociated by repeated aspiration through a sterile Pasteur pipette. Peripheral blood from normal fetuses was obtained by terminal bleeding into sterile PBS. Samples from each day (13, 14, or 16) were pooled and placed on ice. Normal yolk sac cells were collected from 10- and 12-day fetuses by removing Reichert's membrane and dissociating the yolk sac in fresh PBS. Cells were collected by centrifugation (4°C) at $1000 \times g$ for 5 min.

Differential Cell Counts. Slides of dissociated fetal liver cells, yolk sac/blood cells, circulating fetal blood cells, and dissociated spleen cells from phenylhydrazine-treated nor-

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mal adult mice were prepared (Shandon Cytospin 2) and stained with Wright/Giemsa reagents with an automated Hemastainer (Miles, Dallas). At least 300 cells per slide were classified by conventional morphological criteria (28) and the classification system of Kelemen *et al.* (30). Reticulocytes were identified by the new methylene blue method (31).

Hybridization Probes. Transcripts of erythroid ankyrin (*Ank-1*) were detected with a 4.6-kilobase (kb) murine erythroid ankyrin cDNA (mAnk-1; ref. 32). mAnk-1 was specific for the erythroid ankyrin as demonstrated by tests in which mRNAs from the ankyrin-deficient *nb/nb* and *+/+* reticulocytes were analyzed on Northern blots. The ankyrin cDNA used showed normal levels of ankyrin mRNA in reticulocytes from normal mice but greatly reduced levels in *nb/nb* reticulocytes (33). Erythroid spectrin transcripts were detected with a 700-base-pair (bp) murine β -spectrin cDNA (pB58; ref. 34) and a 750-bp murine α -spectrin cDNA (pB129; ref. 35). The specificity of both the α - and the β -spectrin cDNA for the erythroid transcript was demonstrated by Northern analysis of the α -spectrin-deficient *sph^{1J}/sph^{1J}* and β -spectrin-deficient *ja/ja* reticulocyte mRNA, respectively (10, 11).

Northern Blots. Total RNA from fetal erythroid cells and from phenylhydrazine-treated adult spleen cells was prepared by the acid guanidinium thiocyanate/phenol/chloroform method (36). Poly(A)⁺ RNA was isolated by oligo(dT)-cellulose chromatography (37). RNA was electrophoresed in denaturing agarose/formaldehyde gels and transferred to nylon filters (38). Probes were labeled with ³²P by the random hexamer method (39). Hybridizations were performed as specified by Zetabind nylon filters (AMF Cuno) except that 1% SDS was used. After hybridization, filters were washed and exposed to Kodak XAR-5 film at -70°C with an intensifying screen. RNA sizes were determined relative to 18S and 28S RNA.

Immunoprecipitation and SDS/PAGE of Newly Synthesized α - and β -Spectrin. Cells were washed at 37°C in minimal essential medium (MEM) without methionine (16-222-49; Flow Laboratories). An aliquot containing either 10⁸ 16-day fetal reticulocytes or 10⁷ 12-day fetal liver cells was incubated for 90 min at 37°C in 300 μ l of MEM containing 200 μ Ci (7.4 MBq) of [³⁵S]methionine (1186 Ci/mmol; NEG-009A; New England Nuclear) and 10% fetal bovine serum in a 5% CO₂/95% air atmosphere. The cells were washed extensively in medium, lysed in 1% Nonidet P-40, and centrifuged at 780 $\times$ g to remove nuclei. The supernatant, containing membranes and cytoplasmic material, was added to 300 μ l of PBS

containing 0.03 mM phenylmethylsulfonyl fluoride (P-7626; Sigma) and 0.05% SDS and incubated with 30 μ l of antibody for 1 hr at room temperature with gentle agitation. The specificity of the spectrin antibody for the erythroid spectrin and quantitation of the precipitated spectrin monomers have been described (27). The antibody recognized equivalent amounts of α - and β -spectrin monomers and the concentration was adjusted for complete precipitation of the solubilized spectrins. The conjugate was precipitated by incubation with protein A-Sepharose CL-4B (Pharmacia; 100 mg/ml in PBS) for 1 hr at room temperature. The precipitate was washed four times in PBS and the final pellet was dissolved in 50 μ l of SDS sample buffer, boiled for 2 min, and centrifuged to pellet the Sepharose. The supernatant was loaded on a denaturing polyacrylamide gel.

SDS/PAGE (40) was done with a 5% stacking gel and a 10% running gel. The gels were soaked in En³Hance, washed, dried, and exposed to Kodak XAR-5 film at -70°C. Autoradiographs were scanned with a Bio-Rad 620 video densitometer and the areas under the α - and β -spectrin peaks were determined with a Bio-Rad 3392A integrator. The α/β synthetic ratio was calculated from these areas.

RESULTS

Erythroid Precursors in Fetal Erythrogenic Tissue.

During murine ontogeny, the yolk sac is the initial site of erythropoiesis and produces large nucleated cells (28). Cells of yolk sac origin are present in the circulation from days 8–14 but their frequency decreases as smaller nonnucleated cells produced in the fetal liver are released into the circulation. The liver is an active site of erythropoiesis from 11 days of gestation to birth but its contribution to the peripheral blood is limited until after day 12. Maternally derived erythrocytes in the fetus are small, enucleated cells and are easily discernible from yolk sac cells but not from liver-derived cells.

For our purposes it was necessary to identify and quantify the erythroid precursors. Fetal erythroblasts were stained and differential counts were performed. The results are summarized in Table 1 and cells characteristic of the developing fetuses are shown in Fig. 1. Primitive nucleated erythroblasts derived from the yolk sac were present from day 10 to day 14. At day 10, >95% of the nucleated cells were pre-type 1 erythroblasts, and by day 13, the cells matured to type 3 cells. There were $\approx$ 15% maternal cells detected from 10 to 13 days of gestation but, since these cells were not

Table 1. Types of cells present in erythroid compartments

Compartment	Nucleated cells		Nonnucleated cells	
	%	Classification	%	Classification
Yolk sac				
10 days	85	>95% pre-type 1 erythroblasts	15	Maternal red cells
12 days	85	>95% types 1, 2 erythroblasts	15	Maternal red cells
Blood				
13 days	86	>95% type 3 erythroblasts	14	Maternal red cells
16 days	1	Not classified	99	Fetal reticulocytes
Liver				
12–14 days	82	80% pro- and basophilic erythroblasts, 20% polychromatophilic and orthochromic erythroblasts	18	Fetal reticulocytes
Spleen				
Adult*	75	70% pro- and basophilic erythroblasts, 9% polychromatophilic and orthochromic erythroblasts, 21% leucocytes	25	Adult reticulocytes

At least 300 cells from each of three to five animals were classified. Results are expressed as the percent of the total number of cells present. Reticulocytes were enumerated after exposure to new methylene blue stain.

*Phenylhydrazine-treated.

FIG. 1. Cell types characteristic of fetal erythroid compartments. (A) Ten-day yolk sac pre-type 1 erythroblasts. (B) Twelve-day yolk sac type 1 (arrows) and type 2 (arrowhead) erythroblasts. (C and D) Thirteen- and 14-day type 3 erythroblasts. (E and F) Twelve- and 14-day fetal liver erythroblasts; part of a yolk sac cell is seen in E (arrow). (G) Sixteen-day fetal peripheral blood cells; new methylene blue stain revealed that >95% were reticulocytes (data not shown). ($\times 750$.)

reticulocytes, they did not contribute mRNA or newly synthesized protein. By day 14, the nucleated yolk sac cells were diluted with enucleated, liver-derived reticulocytes. The nucleated fetal liver erythroblasts from days 12 to 14 were primarily pro- and basophilic erythroblasts, although more mature polychromatophilic and orthochromic erythroblasts and circulating enucleated erythrocytes were also present. Less than 2% of the cells in the fetal liver were circulating yolk sac cells, and there were no identifiable granulocytes. By 16 days of gestation, the peripheral blood population consisted primarily of liver-derived reticulocytes.

For comparison with the fetal cells, spleen cells from phenylhydrazine-treated adults were also classified. There was a marked expansion of early erythropoietic precursors but, as shown previously in phenylhydrazine-treated rats (21), a significant proportion of nonerythroid cells was also present. The results confirmed that progressively more mature erythroblasts derived first from the yolk sac and later from the fetal liver are present at successive days of gestation (28).

Stage-Specific Expression of *Ank-1* Transcripts. Previously, we reported the differential expression of two erythroid ankyrin (*Ank-1*) transcripts of 9.0 and 7.5 kb in phenylhydrazine-treated adult spleen and reticulocytes (33). The 9.0/7.5-kb ratio was approximately 1:2 in the more immature cells of the highly erythroid spleen. The 7.5-kb message was the dominant form in reticulocytes, where the 9-kb message was barely detectable. These results suggested that the 9-kb transcript might be expressed in the less mature erythrocyte precursors. Hence, the transcripts generated *in vivo* from erythroid compartments of developing fetuses were characterized. Two transcripts of 9.0 and 7.5 kb were detected in fetal liver and yolk sac-derived fetal erythropoietic cells, and

FIG. 2. Northern blots of RNA probed with mAnk-1 cDNA to detect erythroid ankyrin transcripts. (A) RNA from phenylhydrazine-treated adult B6+/+ mice. Lane 1, 10 μ g of total RNA from spleen; lane 2, 4 μ g of poly(A)⁺ RNA from reticulocytes. Note: it was necessary to use polyadenylated reticulocyte RNA to clearly demonstrate the 9.0-kb message. (B) RNA from cells isolated from normal B6+/+ fetal erythropoietic compartments. Lane 1, 5 μ g of 12-day fetal liver total RNA; lane 2, 15 μ g of 16-day fetal reticulocyte total RNA; lane 3, 10 μ g of 10-day yolk sac RNA; lane 4, 10 μ g of 12-day yolk sac RNA; lane 5, 15 μ g of 13-day fetal peripheral blood RNA; lane 6, longer exposure of lane 3 showing the 9.0- and 7.5-kb transcripts in pre-type 1 yolk sac erythroblasts. All lanes were from the same gel and were hybridized simultaneously. The lanes were repositioned to show increasingly mature cells from left to right.

these fetal *Ank-1* transcripts comigrated with those detected in adults (Fig. 2). The 9.0/7.5-kb ratio was 1:1 in the less mature cells from 10-day yolk sac and 12-day fetal liver. Similar results were obtained in 14-day fetal liver (data not shown). Total cellular RNA isolated from the more differentiated peripheral blood reticulocytes of 16-day fetuses showed only the 7.5-kb transcript, although small amounts of the 9.0-kb transcript were detected in poly(A)⁺ RNA (data not shown). This pattern is identical to that seen in adult reticulocytes (33). Similarly, where successively later stages of primitive erythroblasts were present, as in the progression from pre-type 1 yolk sac erythroblasts at 10 days to types 2 and 3 at 12 and 13 days, the 7.5-kb transcript became the major species. Hence, in the erythroid populations tested, the early erythroid precursors from 10-day yolk sac, fetal liver, and erythrogenerative spleen expressed more of the 9.0-kb RNA than did the more mature cells from the 12/13-day yolk sac, 16-day fetal peripheral blood, and adult reticulocytes. In the human, the 9.0- and 7.2-kb transcripts coexist in all erythroid tissues examined, and the ratio seems to decrease with differentiation in a fashion similar to that seen in the mouse (41).

Based on the amount of *Ank-1* mRNA relative to the total amount of RNA applied to the gels, expression of *Ank-1* was highest in fetal liver erythroblasts and decreased in fetal reticulocytes (Fig. 2). The opposite was true of the yolk sac-derived lineage; *Ank-1* expression was lowest in the earliest precursors (10-day yolk sac) but increased in more mature erythroblasts from days 12 and 13 of gestation.

Expression of Spectrin Transcripts During Erythropoiesis. There were two β -spectrin transcripts, 10.1 and 9.3 kb, detected in the spleens and reticulocytes of phenylhydrazine-treated adult mice (Fig. 3). The 10.1-kb transcript was present at twice the level of the 9.3-kb transcript. Examination of the fetal transcripts showed the following results. Unlike ankyrin, no stage-specific expression of β -spectrin mRNAs was evident; the 10.1/9.3-kb ratio did not change in adult (Fig. 3) vs. fetal cells (Fig. 4) nor in immature vs. more mature precursors (Fig. 4). The amount of β -spectrin transcript

FIG. 3. Northern blot of spleen and reticulocyte RNA from phenylhydrazine-treated adult mice. The Northern transfers were probed with pB58 to detect β -spectrin transcripts in 8 μ g of total RNA from spleen (lane 1) and reticulocytes (lane 2).

FIG. 4. Northern blot of total RNA isolated from B6-+/+ fetal erythroid compartments. A single blot was hybridized first with pB129 to demonstrate α -spectrin transcripts (B), then stripped and subsequently probed with pB58 to demonstrate β -spectrin transcripts (A). All hybridization and wash conditions were identical. The α -spectrin filter was exposed for 4 days, β -spectrin for 3 days. Lane 1, 10 μ g of 12-day fetal liver; lane 2, 15 μ g of 10-day yolk sac; lane 3, 15 μ g of 12-day yolk sac; lane 4, 20 μ g of 16-day reticulocytes.

relative to the total RNA concentration increased during yolk sac differentiation but decreased during differentiation of the liver precursors to reticulocytes in a pattern similar to *Ank-1* mRNA.

An α -spectrin transcript of 10.2 kb was detected in phenylhydrazine-treated adult spleen and reticulocytes (data not shown) and was also present in all fetal cells, except the 10-day yolk sac cells (Fig. 4). The relative amount of the α -spectrin transcript increased during differentiation of both the yolk sac- and fetal liver-derived erythroblasts. Interestingly, the amount of α -spectrin transcript relative to the total RNA was significantly lower than the amount of β -spectrin transcript in the early erythropoietic precursors from both yolk sac and fetal liver. This was not due to differences in experimental conditions, since the α -spectrin and β -spectrin cDNA probes were similar in size (750 bp and 700 bp, respectively) and were labeled to the same specific activities (10^8 cpm/ μ g of DNA), and the hybridization and wash conditions were identical. The same Northern transfer filter was exposed for 4 days to show α -spectrin transcripts and 3 days to show β -spectrin transcripts. This pattern of increased β -spectrin mRNA expression over α -spectrin mRNA in early erythropoietic precursor cells was consistent with the $\alpha < \beta$ spectrin protein synthesis observed in erythropoietin-stimulated Friend virus-infected murine spleen cells (24) but unlike the protein synthetic profile seen in reticulocytes from phenylhydrazine-treated adult mice (27) and rats (21).

Immunoprecipitation of Newly Synthesized α - and β -Spectrin in Fetal Erythroid Cells. To determine whether the mRNA levels were indicative of protein synthetic patterns, total newly synthesized α - and β -spectrin were quantified in 12-day fetal liver erythroblasts and 16-day fetal reticulocytes. Cells were incubated in the presence of [35 S]methionine, lysed, and precipitated with an antibody that recognizes equivalent amounts of α - and β -spectrin on Western blots (27). In 12-day fetal liver, total β -spectrin synthesis was 3-fold greater than that of α -spectrin (α/β synthetic ratio = 0.33) (Fig. 5). In 16-day fetal reticulocytes, however, total α -spectrin synthesis was 2-fold greater than β -spectrin synthesis (α/β synthetic ratio = 2.0). In this regard, 16-day fetal reticulocytes were similar to phenylhydrazine-treated mouse reticulocytes (27). Hence, total α - and β -spectrin synthesis correlates with the Northern blot data.

DISCUSSION

The relevant observations from this study are that (i) the 7.5-kb ankyrin mRNA, while present in the less mature erythroblasts from all erythrogenic tissues tested, becomes the major transcript in the more mature erythroblasts;

FIG. 5. Immunoprecipitation of total newly synthesized spectrin. Cells were metabolically labeled with [35 S]methionine and immunoprecipitated with spectrin antibody. Lanes 1 and 2, 12-day fetal liver cells (10^7); lane 3, 16-day fetal reticulocytes (10^6). The shift in the α/β synthetic ratio from 12-day fetal liver erythroblasts to 16-day fetal reticulocytes is apparent.

(ii) there is more β - than α -spectrin mRNA in yolk sac-derived nucleated cells and in the fetal liver erythroblasts, but the converse occurs in the more mature fetal liver-derived and adult reticulocytes; and (iii) the relative amount of mRNA for each cytoskeletal protein is dependent not only on the stage of differentiation but also on the source of the erythroblasts. The observed mRNA changes are summarized in Table 2 and, since we have shown that the amount of α - and β -spectrin synthesized is consistent with mRNA levels, also predict the protein synthetic ratios.

Regardless of cell origin (fetal yolk sac, fetal liver, adult spleen), our results indicate that ankyrin mRNA is differentially processed depending on the stage of erythroid maturation. We find nearly equivalent levels of 9.0- and 7.5-kb mRNA in the early cells, and predominance of the 7.5-kb mRNA in later stages of erythroid cell differentiation. Although these results support our contention (33) that the 9-kb mRNA generates a product essential for structural integrity of the early erythroid cell membrane skeleton, the situation is probably more complex. Structural analyses of ankyrin and protein 4.1 cDNAs indicate that alternative splicing of multiple small exons can produce many transcripts from a single gene (41–46) that are structurally different but comigrate during electrophoretic separation. Therefore, the apparent 9.0- to 7.5-kb switch of ankyrin mRNA during erythroid development could involve several 9.0- and/or 7.5-kb transcripts designed to produce proteins with minor structural changes but significant changes in binding properties. Ankyrin isoforms display different β -spectrin and anion exchanger binding affinities (47) and bind alternative proteins in the erythroid membrane (48, 49). It is possible that a closer examination of transcripts from the erythroid α - and β -spectrin genes may reveal similarly complex patterns of splicing. As extensive remodeling of the erythrocyte membrane occurs during erythropoiesis (50, 51), it is reasonable to propose that stage-specific mRNA splicing produces isoforms of the

Table 2. Summary of transcript characteristics

Cell source	Major cell type*	Transcripts†			
		Ankyrin		Spectrin	
		9/7.5 kb	Amount	β	α
Yolk sac	Pre-1	1	+	+	ND
	1, 2	<0.1	++	++	+
	3	<0.1	+++	+++	++
Fetal liver	Pro, Baso	1	+++	+++	+
	Retic	<0.1	+	+	++
Spleen	Pro, Baso	0.5	—	—	—
	Retic	<0.1	—	—	—

*Pro, proerythroblast; Baso, basophilic erythroblast; Retic, reticulocyte. Refer to Table 1 for time of appearance of cell types.

†For β -spectrin, the ratio of the 10.1- and 9.3-kb transcripts was 2 in all samples. For α -spectrin, only a 10.2-kb mRNA was seen. ND, not detectable; —, no data.

spectrin-binding proteins, ankyrin and 4.1, that function to meet membrane requirements unique to each developmental stage.

Heterodimers of α - and β -spectrin are incorporated stoichiometrically (1:1) into the membrane despite the fact that unequal amounts of the two proteins are synthesized (21, 27). Expression of α - and β -spectrin transcripts and the α/β protein synthetic ratio can vary significantly depending upon physiological conditions. For example, in patients with ankyrin-deficient hemolytic anemia (8), in some dimethyl sulfoxide-induced murine erythroleukemia cells (18, 22), and in erythropoietin-stimulated, Friend virus-infected murine spleen cells (24), β -spectrin expression and synthesis are increased over those of α -spectrin ($\alpha/\beta < 1$). In adult reticulocytes, the α/β synthetic ratio is usually 2–3:1. The altered α/β synthetic ratio produced by erythropoietin stimulation increases β -spectrin mRNA expression and synthesis without affecting that of α -spectrin. This phenomenon results in increased membrane spectrin incorporation (24). In the present study, higher levels of β -spectrin mRNA than α -spectrin mRNA and increased β -spectrin synthesis relative to α -spectrin synthesis were found in early erythroid precursors of the fetal liver. The cells showing increased β -spectrin mRNA expression and protein synthesis in this study are known to be erythropoietin-sensitive (pro- and basophilic erythroblasts) (23). The increased expression and synthesis of β -spectrin seen in these early erythroid precursors suggest increased membrane demand for spectrin tetramers, most likely reflecting the dynamic nature of the differentiating erythrocyte membrane. Physiological control of the α/β synthetic ratio may provide an important mechanism for maintaining the erythroid cell membrane during erythropoiesis.

The erythrocyte *in vivo* possesses two putative mechanisms to meet varying membrane binding and/or mechanical properties during differentiation: (i) generation of stage-specific ankyrin and protein 4.1 isoforms by alternative mRNA processing and (ii) the ability to alter α/β -spectrin synthetic ratios. In the future, more detailed analyses must include the use of region-specific antibodies (52) and oligonucleotide probes (53). Only when unique cytoskeletal products are identified and their interactions with other proteins are established will we begin to understand the process of erythroid cell differentiation.

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