**BRIEF REPORT**

**Embryonic Hemoglobins Are Expressed in Definitive Cells**


Human embryonic ζ and ε globin chains are synthesized in yolk sac–derived primitive erythroid cells, and decrease rapidly during definitive erythropoiesis. Examination of ζ and ε globin expression at the cellular level using dual-color immunofluorescence staining with specific monoclonal antibodies showed that embryonic globin proteins are present in definitive erythrocytes. More than half of fetal erythrocytes were positive for ζ and ~5% for ε globin. Approximately one third of newborn red blood cells were ζ-positive and less than 1% ε-positive. Adult erythrocytes did not have embryonic globins. Erythroblasts that developed in liquid cultures also contained embryonic globin in amounts which declined with ontogenic age, and the proportion of positive cells in vitro was less than in the comparable erythrocytes that developed in vivo. Thus, embryonic globin chains are synthesized in definitive erythrocytes and decrease with ontogeny. Modulation of embryonic globin gene expression is not solely due to a switch from primitive to definitive erythropoiesis.

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cytes, significantly fewer than in fetal samples. Five of six cord samples had 0.6 ± 0.5% ε+ or ζ+/ε+ erythrocytes (Fig 1B). In cultures from three cord blood samples 4% ± 1% of the erythroblasts were ζ+ and rare cells were ε+ (Fig 1E). Thus, embryonic globins are clearly present in erythroid cells at term birth. There were no embryonic-positive adult erythrocytes (Fig 1C), and cultured adult erythroblasts positive for ζ or ε comprised 0.2% of the cells in only 1 of 7 experiments (Fig 1F).

The ontogenic decline in the proportion of embryonic-positive cells is documented in Fig 2. The differences between the percent ζ+ cells in fetal, cord, and adult erythrocytes and erythroblasts are significant at \( P < .001 \). The data for the expression of ε globin are also compelling. The amount of embryonic globin per cell appears qualitatively to decrease during ontogeny.

The \( \alpha \) and non-\( \alpha \) globin gene clusters may be regulated differently. The non-\( \alpha \) cluster consists of ε, γ, Aγ, ψβ, δ, and β genes and has embryonic, fetal, and adult stages. The locus control region (LCR) 5 to 20 kb upstream from ε has open chromatin only in erythroid cells, and ε gene regulation is autonomous. In transgenic mice, ε is expressed only in primitive erythroid cells and undergoes developmental silencing in definitive cells. With a yeast artificial chromosome (YAC) construct of ε, γ, and β genes, all primitive cells contained γ RNA, although many also had ε RNA, which is consistent with sequential or simultaneous transcription of the globin genes. In definitive cells, developmental stage-specific transacting factors may affect the interaction of the LCR with γ or β, but not with the ε gene.23-26 Our single cell data suggest that ε silencing may be leaky, because a few definitive cells in fetal and newborn blood were strongly positive for ε globin protein.

The \( \alpha \) cluster consists of a DNAse I hypersensitive region

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**Fig 1.** Embryonic globin protein detection with dual color immunocytofluorescence staining. Samples were from 22-week-old fetus, term cord, and adult blood. Blood smears or cytospins were stained with MoAbs specific for ζ and ε globins conjugated with FITC and TR, respectively. (A) Fetal, (B) cord, and (C) adult erythrocytes. (D) Fetal, (E) cord, and (F) adult cultured erythroblasts.

**Fig 2.** The percent of cells that were positive for embryonic globins in erythrocytes and cultured erythroblasts. Left, ζ+ cells. Right, ε+ cells. RBC, red blood cells; Culture, day 14 of culture; F, fetal; C, cord; A, adult. Note the difference in the y-axis scales for the two embryonic globins. There is an ontogenic decline in the percent positive cells, both in vivo and in culture.
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(HS-40), which is 40 kb upstream, followed by ζ2, ψζ1, ψα2, ψα1, α2, α1, and θ genes. Unlike the β LCR, the α HS has open chromatin in both erythroid and nonerythroid cells. Developmental silencing of ζ is regulated by synergy between the 5′ ζ promoter and 3′ flanking sequences, with an additional autonomou

We have documented embryonic globin chains in definitive cells, a developmental decline in the proportion of positive cells, and an apparent decrease in the amount per cell. ε expression decreases more rapidly than ζ, perhaps related to ε transcription autonomy, and ζ is modulated at both transcriptional and posttranscriptional levels. One reason for these differences may be that there is a clear fetal stage for non-α genes (ie, γ), but there is not one for α genes. The persistence of ζ-gene expression in fetal definitive cells may be comparable to the appearance of γ globin chains.

REFERENCES
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