

Fetal Hemoglobin Synthesis in Erythroid Cultures in Hereditary Persistence of Fetal Hemoglobin and $\beta^a$-Thalassemia

By Rona S. Weinberg, Stylianos E. Antonarakis, Haig H. Kazazian, Jr., George J. Dover, Stuart H. Orkin, Alexandra L. Lenes, J. Matthew Schofield, and Blanche P. Alter

To determine whether hemoglobin regulation is normal in diseases affecting $\beta$-globin gene expression, globin synthesis was examined in members of a family with hereditary persistence of fetal hemoglobin/$\beta^a$-thalassemia (HPFH/$\beta^a$-thal). The HPFH defect is the Ghanian type II, with a deletion from $\psi\beta$, at least 20 kb 3' to $\beta$. The $\beta^a$-thal gene has the haplotype II restriction enzyme pattern and has the $\beta 39$ nonsense mutation. Erythroid colonies from blood BFU-E were radiolabeled, and globin chains were separated by gel electrophoresis. Colonies from the $\beta^a$-thal heterozygote had non-\(a/\alpha\) ratios more balanced with a deletion from $\psi\beta$. Hereditary persistence of fetal hemoglobin/\(\beta\)-thalassemia (HPFH/$\beta^-\text{thal}$) was examined in members of a family of a patient with diseases affecting $\beta$-globin gene expression. $\beta\text{-globin}$ synthesis was 11% of $\beta^-\text{thal}$ heterozygote had non-\(a/\alpha\) ratios more balanced with a deletion from $\psi\beta$.

Patients with hemoglobinopathies often have increased levels of hemoglobin F (HbF) in vivo. Investigation of such patients at the molecular and cellular levels may contribute to our understanding of the regulation of hemoglobin control and may eventually permit therapeutic manipulation of HbF production. We have studied several members of a Puerto Rican family in which the genes for pancellular hereditary persistence of fetal hemoglobin (HPFH) and $\beta$-thalassemia ($\beta^-\text{thal}$) are present in various combinations. We characterized both of the $\beta$-globin gene mutations and examined their effect on HbF synthesis in vivo and in vitro.

HbF synthesis appears to be a normal component of adult erythroid development. The evidence for this was obtained in cultures derived from erythroid progenitor cells in semisolid media. Early progenitor cells (BFU-E: burst-forming units, erythroid) produce cob-

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Hb SYNTHESIS IN CULTURE

The normal ontogenic switch from HbF to HbA was affected globin synthesis by these adult colonies.23-24

We now wished to determine whether increased HbF in adults in vivo derives from reactivation of fetal progenitors or augmentation of the HbF program of adult progenitors. We would expect high HbF and correlation of (γ) and γ levels in erythroid colonies in vitro if the fetal progenitor were involved. Less high HbF and no correlation of Gγ and γ would indicate adult progenitor-derived programs. To determine which type of progenitor cell could be responsible for the increased HbF seen in individuals with β-chain hemoglobinopathies, we examined globin synthesis in individual colonies from thalassemic and HPFH heterozygotes in the family presented in this article.

MATERIALS AND METHODS

Blood Studies

Peripheral blood was obtained from the antecubital vein of each individual. All procedures were approved by the Research Advisory Committee of the Mount Sinai School of Medicine. Samples were collected into syringes containing heparin (Liquaemin, Organon Inc., West Orange, NJ). 50 U/ml blood, as well as in EDTA tubes. Blood counts were obtained with a Coulter Model S, and cellular distribution of HbF was examined by the acid elution technique25 using a kit from Boehringer-Mannheim (Indianapolis, IN). The percent F cells and the concentration of HbF per F cell was determined by radial immunodiffusion assay with anti-HbF.26

DNA Analyses

High molecular weight DNA was isolated from the leukocytes of 10-15 ml of EDTA-treated peripheral blood from all members of the family as described.27 Five to ten micrograms of DNA from each individual was digested overnight with one of various restriction endonucleases, using conditions recommended by the commercial suppliers. The resulting DNA fragments were separated according to size by electrophoresis in 0.8%-1.0% agarose gels, transferred to nitrocellulose membranes, fixed, and hybridized with 32P-labeled cDNA fragments,35 (4) a 0.7-kb genomic Bgl II-Eco RI fragment located 17 kb 3' to the β-globin gene,36 (9) a 1.55-kb Sst I genomic DNA fragment37 that contains sequences approximately 4.5 kb 3' to the end of the Ghanian HPFH deletion (HPFH-II)38,39 and (6) a cDNA fragment of plasmid JW101 containing human α-globin gene sequences.34 All fragments were radiolabeled with 32P-dATP and 32P-dCTP by the nick translation function of Escherichia coli DNA polymerase I, as described.40

The thalassemic defect was identified by hybridization with an oligonucleotide probe (a 19-mer called 19T) with the sequence CTTTGACCTAGGTTCT, which is specific for the B39 nonsense mutation. Bam HI digested DNA was hybridized at 50°C and washed stringently, with the final wash at 56°C, using procedures described previously.41

Erythroid Cultures

Peripheral blood mononuclear cells were centrifuged on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) and plated at 3 x 10^6 cells/ml in 0.3 ml methylcellulose cultures for 14 days, as described previously.20 The erythropoietin was used at 2 U/ml (CAT-1, 1,140 U/mg protein, kindly provided by the Division of Blood Diseases and Resources of the National Heart Lung and Blood Institute). Globin synthesis was studied by the addition of 100 µCi of 3H-leucine to 0.3-ml plates, or by removal of single red bursts and labeling each individually with 50 µCi of 3H-leucine (~100 Ci/mmol, New England Nuclear Corp., Boston, MA). The incubations with 3H-leucine were carried out from day 13 to day 14. Labeling and harvesting were performed as described elsewhere.20 Globin chain synthesis by the erythroid colonies was evaluated by electrophoresis on 0.8-mm thick polyacrylamide slab gels containing acetic acid, urea, and Triton X-100.42 Fluorography and evaluation of relative globin chain syntheses were also described.34,44,45 Analyses of triplicate samples indicate that data are accurate to ±3%.46 The percent γ synthesis was calculated from 32P/3H (γ + Gγ) x 100, and percent γ synthesis was calculated from γ/(γ + β) x 100. The observed distributions of γ and γ in each group of single colonies were plotted. That the observed data are samples from normal distributions was tested using the W statistic.47 Nonparametric (Spearman's) rank order correlation coefficients (r) were determined, as were linear regressions when values were normally distributed. p Values ≤0.05 are considered to be statistically significant and to suggest correlation between γ and γ syntheses. Analyses of the results were made with PROPHET, a time-sharing computer facility sponsored by the Division of Research Resources of the National Institutes of Health.

Case Reports

The propositus, a 6-yr-old Puerto Rican female, was referred for evaluation of splenomegaly. She was found to be short (~10th percentile), icteric, and had thalassemic facies, a 2/6 systolic flow murmur, a 7-cm spleen, and a 1-cm liver. Her hematologic findings are shown in Table 1. Specifically, she had moderate anemia, microcytosis, reticulocytosis, and approximately 100% HbF and, thus, appeared to have thalassemia intermedia. Data on other family members are also shown in Table 1. Because her mother and father are heterozygous for pancellular HPFH and β-thalassemia, respectively, the propositus is a double heterozygote for HPFH and β-thalassemia. The HPFH gene in this family is pancellular, because 100% of the cells of the HPFH heterozygotes contain HbF, both by acid elution and by radial immunodiffusion. The β-thalassemia gene is β, because the patient has neither HbA protein nor β-globin chain synthesis. Her clinical status is more severe than previously reported HPFH/β-thal patients,46 as they had mild black β'-thal genes, whereas hers is a severe Mediterranean β'-gene.

RESULTS

DNA Analyses

α-Globin Gene Cluster

There was no deletion of DNA sequences in the α-globin gene cluster. After Bam HI digestion of...
Table 1. Hematologic and Culture Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mother</th>
<th>Father</th>
<th>Sibling 1</th>
<th>Propositus</th>
<th>Sibling 2</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>HPFH*</td>
<td>β-thal*</td>
<td>Normal</td>
<td>HPFH/β-thal*</td>
<td>HPFH*</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>34</td>
<td>33</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.2</td>
<td>14.2</td>
<td>13.4</td>
<td>8.4</td>
<td>13.0</td>
<td>13–14</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>84.3</td>
<td>61.3</td>
<td>84.0</td>
<td>66.5</td>
<td>78.2</td>
<td>85–100</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28.1</td>
<td>20.2</td>
<td>28.6</td>
<td>22.8</td>
<td>26.5</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>0.5</td>
<td>1.2</td>
<td>0.8</td>
<td>4.1</td>
<td>0.5</td>
<td>0–2</td>
</tr>
<tr>
<td>RBC size CV (%)</td>
<td>16.6</td>
<td>16.7</td>
<td>15.0</td>
<td>48.4</td>
<td>16.7</td>
<td>14.9 ± 0.8</td>
</tr>
<tr>
<td>Betke positive (%)</td>
<td>100</td>
<td>3.0</td>
<td>1.0</td>
<td>100</td>
<td>100</td>
<td>&lt;1</td>
</tr>
<tr>
<td>F cells (%)</td>
<td>100</td>
<td>3.5</td>
<td>2.6</td>
<td>100</td>
<td>100</td>
<td>0.4–4.5</td>
</tr>
<tr>
<td>HbF (pg/F cell)</td>
<td>4.6</td>
<td>—</td>
<td>0.2</td>
<td>22.2</td>
<td>4.4</td>
<td>4–5</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>27.2</td>
<td>0.2</td>
<td>0.2</td>
<td>97.3</td>
<td>26.0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>6Gγ (%)</td>
<td>38</td>
<td>—</td>
<td>—</td>
<td>55</td>
<td>38</td>
<td>40–60</td>
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<tr>
<td>HbA2 (%)</td>
<td>2.2</td>
<td>6.0</td>
<td>3.2</td>
<td>2.0</td>
<td>3.0</td>
<td>2.5–3.5</td>
</tr>
<tr>
<td>Titer</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1250</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Colonies/10⁵ cells</td>
<td>6 ± 2</td>
<td>11 ± 5</td>
<td>10 ± 3</td>
<td>15 ± 8</td>
<td>2 ± 1</td>
<td>5–40</td>
</tr>
</tbody>
</table>

Table 1. Hematologic and Culture Data

*These individuals are heterozygotes; the propositus is a double heterozygote.

The haplotypes for various restriction site polymorphisms in the globin gene cluster of the father's β-thalassemic gene are shown in Fig. 1B. The following polymorphic restriction sites were analyzed: Hind III sites in the IVS-2 of Gγ  and γ-globin genes, Hinc II sites in and 3' to the ψβ-globin gene, Hgi Al site in the first exon of the β-globin gene, Ava II site in the IVS-2 of the β-gene, and Barn HI site 3' to the β-gene. The paternal β-thalassemia chromosome is that of haplotype II, associated with the pattern [+++−β+++], which was found in 17% of 91 Mediterranean chromosomes and was associated in that population with a nonsense mutation at codon 39. The direct hybridization with the synthetic oligonucleotide specific for that region identified the β39 nonsense mutation in this family.

Erythroid Colonies

In all 5 individuals, blood BFU-E-derived colony growth resembled that of normals and was maximal on day 14. The plating efficiencies for each family member ranged from 2 to 15 colonies/10⁵ cells plated (Table 1).

Globin chain synthesis was examined in whole plates and individual colonies (Table 2). Only red, relatively mature colonies were picked to avoid the confounding factor of asynchronous globin synthesis during colony and erythroblast maturation. Differences between values in whole plates and in the means of single colonies reflect the influence on whole plate data of the most highly biosynthetically active and hence radioactive colonies and thus indicate the presence of biosynthetic variability due to maturational differences in individual colonies. The single colonies picked in each study may not have been entirely representative of all colonies present in whole plates. All single colonies

Figure 1. (A) Schematic representation of the β-globin gene deletion in the HPFH individuals. The 5' end of the deletion lies between the Bgl II and Xba I sites and is indicated by a dashed line (---). The 3' end of the deletion is at an unknown distance greater than 20 kb 3' to the β-globin gene. This deletion has been previously reported as the Ghanian or type II HPFH deletion.36–38 Figure 1A shows the extent of the deletion as revealed by several different endonuclease digestsions of DNA and hybridization of the resulting fragments with known probes.

Figure 1. (A) Schematic representation of the β-globin gene deletion in the HPFH individuals. The 5' end of the deletion lies between the Bgl II and Xba I sites and is indicated by a dashed line (---). The 3' end of the deletion is approximately 20 kb 3' to the β-gene. (B) Schematic representation of the restriction site polymorphisms examined in the β-thalassemia gene. The pattern is haplotype II, [+++−β+++].
produced some γ. Representative spectrophotometric scans and fluorograms of individual colonies from the mother (A), father (B), and propositus (C), are shown in Fig. 2. γ-synthesis was 29%, 62%, and 51% in the colonies in A, B, and C, respectively, while total γ-synthesis in each was 8%, 7%, and 100%. There was no β-synthesis in any colonies from the propositus, C. Nonglobin protein synthesis was not significant.

The biosynthetic ratio of non-α/α-globin proteins was higher, and thus more balanced, in the BFU-E-derived colonies than in the reticulocytes of each individual (Table 2). The γ/α synthetic ratio was not changed in the erythroid colonies of the HPFH individuals, but was increased in colonies compared to reticulocytes from the other family members. The β/α synthetic ratio was increased in erythroid colonies of all donors, except, of course, the propositus, who did not synthesize any β-chains in vivo or in vitro. The proportion of γ-chain synthesis (of β + γ) was elevated in colonies compared to reticulocytes in the β-thal heterozygote and normal, but was not increased in the two HPFH heterozygotes, despite an increase in the non-α/α ratio.

The comparisons of γ and γ-synthesis in single erythroid colonies from each family member are shown in Table 2 and Figs. 3 and 4. As explained above, only red, mature, well hemoglobinized colonies were studied in each experiment. The mean γ-synthesis was normally distributed in colonies from all donors. The approximate range of γ was 20%-70% of total γ-synthesis. Mean γ, as a proportion of non-α-synthesis in single colonies, was 6% in the normal, 18% and 23% in the two HPFH heterozygotes, 11% in the β-thal heterozygote, and 100% in the HPFH/β-thal patient. These values (except for the propositus) are in the

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**Table 2. Globin Chain Synthesis by Reticulocytes and Erythroid Colonies**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Specimen</th>
<th>n</th>
<th>γ/α (Mean ± SD)</th>
<th>β/α (Mean ± SD)</th>
<th>Non-α/α (Mean ± SD)</th>
<th>Percent γ</th>
<th>Percent γ</th>
<th>Spearman’s</th>
<th>Linear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother§</td>
<td>Reticulocytes</td>
<td>1</td>
<td>0.23 ± 0.10</td>
<td>0.74 ± 0.08</td>
<td>1.11 ± 0.05</td>
<td>48 ± 6</td>
<td>2.0 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>HPFH</td>
<td>Whole plates</td>
<td>3</td>
<td>0.37 ± 0.10</td>
<td>0.74 ± 0.08</td>
<td>1.11 ± 0.05</td>
<td>57 ± 6</td>
<td>2.0 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>Singles</td>
<td>20</td>
<td>0.18 ± 0.09</td>
<td>0.79 ± 0.22</td>
<td>0.97 ± 0.16</td>
<td>38 ± 7</td>
<td>2.6 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>Father§</td>
<td>Reticulocytes</td>
<td>1</td>
<td>0.30 ± 0.13</td>
<td>0.95 ± 0.01</td>
<td>1.25 ± 0.12</td>
<td>55 ± 6</td>
<td>0.4 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>β-thal</td>
<td>Whole plates</td>
<td>3</td>
<td>0.20 ± 0.09</td>
<td>0.75 ± 0.15</td>
<td>0.84 ± 0.17</td>
<td>46 ± 6</td>
<td>0.9 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>Singles</td>
<td>24</td>
<td>0.09 ± 0.05</td>
<td>0.64 ± 0.04</td>
<td>0.75 ± 0.15</td>
<td>61 ± 4</td>
<td>2.6 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>Sibling 1</td>
<td>Reticulocytes</td>
<td>1</td>
<td>0.08 ± 0.04</td>
<td>1.10 ± 0.09</td>
<td>1.87 ± 0.08</td>
<td>61 ± 4</td>
<td>5.2 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>Normal</td>
<td>Whole plates</td>
<td>3</td>
<td>0.08 ± 0.04</td>
<td>1.10 ± 0.09</td>
<td>1.87 ± 0.08</td>
<td>61 ± 4</td>
<td>5.2 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>Propositus§</td>
<td>Reticulocytes</td>
<td>1</td>
<td>0.20 ± 0.10</td>
<td>0.15 ± 0.06</td>
<td>0.96 ± 0.09</td>
<td>55 ± 6</td>
<td>0.4 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>HPFH/β-thal</td>
<td>Whole plates</td>
<td>3</td>
<td>0.37 ± 0.32</td>
<td>0.37 ± 0.32</td>
<td>0.37 ± 0.32</td>
<td>55 ± 6</td>
<td>2.6 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
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<tr>
<td>Singles</td>
<td>33</td>
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<td>0.40 ± 0.14</td>
<td>0.40 ± 0.14</td>
<td>61 ± 6</td>
<td>3.3 ± 0.5</td>
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<td>0.05 ± 0.46</td>
<td>0.04 ± 0.04</td>
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<tr>
<td>Sibling 2§</td>
<td>Reticulocytes</td>
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<td>0.12 ± 0.09</td>
<td>0.44 ± 0.04</td>
<td>0.56 ± 0.04</td>
<td>61 ± 6</td>
<td>2.1 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>HPFH</td>
<td>Whole plates</td>
<td>1</td>
<td>0.09 ± 0.09</td>
<td>0.95 ± 0.04</td>
<td>1.04 ± 0.04</td>
<td>61 ± 6</td>
<td>2.1 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
</tr>
<tr>
<td>Singles</td>
<td>12</td>
<td>0.19 ± 0.17</td>
<td>0.62 ± 0.18</td>
<td>0.81 ± 0.25</td>
<td>48 ± 6</td>
<td>2.2 ± 0.5</td>
<td>0.51 ± 0.05</td>
<td>0.05 ± 0.46</td>
<td>0.04 ± 0.04</td>
</tr>
</tbody>
</table>

* Determined by carboxymethylcellulose column chromatography of globin chains.

† Determined by electrophoresis on polyacrylamide slab gels.

$p < 0.05$ suggests correlation of γ and γ-syntheses.

§ These individuals are heterozygotes; the propositus is a double heterozygote.

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**Fig. 2.** Representative spectrophotometric scans and fluorograms of individual colonies from (A) mother (HPFH heterozygote), (B) father (β-thal heterozygote), and (C) propositus (HPFH/β-thal).

**Fig. 3.** Percent γ and γ synthesis by single colonies from (A) mother (HPFH heterozygote), (B) father (β-thal heterozygote), (C) sibling 1 (normal), and (D) sibling 2 (HPFH heterozygote).
range seen in colonies from normal adults. The γ-synthesis was normally distributed in all colonies except those from the normal individual (data not shown). Oγ and γ-syntheses were clearly not correlated in the normal, β⁰-thal, and one of the HPFH individuals (sibling 2), although the correlation was borderline in the other HPFH heterozygote (mother), using both the Spearman’s rank order correlation, $r_s$, and linear regression analysis in those with normal distributions.

**DISCUSSION**

The family described here provided the unique opportunity to examine the effect of β⁰-thalassemia and HPFH defects on globin chain production in vivo and in vitro, separately and in combination. Unlike previous studies of thal and HPFH, we have defined each mutant gene at the molecular level. Although the erythrocytes of such patients are not “fetal,” the HbF program of the progenitor cells might be fetal rather than adult.

In other reports, erythroid colonies derived from β-thalassemic heterozygotes were found to have more balanced non-α/α-synthesis than reticulocytes, due to a relative increase in γ-chain production. This increase in γ-gene expression was thought to be greater than occurs in colonies from normal adults in some patients,¹⁰ but not in others.¹⁴,¹⁶ In the β⁰-thal heterozygote presented here, γ-synthesis was significantly higher in BFU-E-derived colonies than in reticulocytes, but was not above that observed in cultures from normal adults. The β/α ratio was also increased in his colonies compared to his reticulocytes. The more balanced non-α/α-synthesis was thus due only in part to the increased γ-synthesis. We cannot distinguish an increase in β-synthesis from an apparent decrease in α-synthesis due to selective proteolysis of α-chains. This process has been demonstrated in bone marrow erythroblasts⁵⁰ and may be relevant in erythroid cultures as well. Our study of globin synthesis in this β⁰-thal individual thus suggests that increased HbF in vitro occurs by mechanisms similar to those seen in normal adults.

Erythroid colonies from the two HPFH heterozygotes in our study did not produce relatively more γ than did their reticulocytes. This is similar to the two cases studied by Huisman et al.,¹⁶ but is different from the one reported by Papayannopoulou et al.¹⁸ The genetic defects may not be the same in all of these cases. We did observe an increase in the non-α/α ratios in our cases, due to relative increases in both β and γ-syntheses. It is possible that the normal β-globin gene (on the chromosome trans to the HPFH deletion) contributes more to globin synthesis in progenitor-derived colonies than in reticulocytes. However, α-chain proteolysis might also be relevant. In any case, failure to find relatively more γ-synthesis in cultures than in reticulocytes suggests that the HbF program in HPFH does not change between erythroid progenitor cells and their more mature progeny. The region that is deleted in the HPFH chromosome may thus contain sequences responsible for the modulation of γ-gene expression during normal erythroid development.

In the propositus, a double heterozygote for HPFH and β⁰-thal, the non-α/α ratio was also increased in vitro. Because relative synthesis of γ-globin (of β + γ) was increased in colonies compared to reticulocytes from the β⁰-thal, but not the HPFH studies, the increased non-α/α ratio in the patient might be due, at least in part, to increased γ-synthesis from the β⁰-thal chromosome. Here, too, however, the contribution of α-globin proteolysis cannot be excluded. As the colonies and the reticulocytes produced 100% HbF, there is no switch from HbF to HbA that requires explanation.

When single colonies were examined, all colonies from each individual produced some γ-globin, as predicted from single cell immunodiffusion studies of normals.⁵¹ Oγ and γ-syntheses were not correlated in the normal, β⁰-thal heterozygote, or one of the HPFH heterozygotes, although the $p$ value was borderline in the other HPFH carrier. The lack of correlation, plus the adult levels of each of these parameters, suggest that their erythroid progenitors were of the adult, not the fetal type.²⁰

Our studies of this family with HPFH and β⁰-thal genes provide information regarding HbF regulation in the context of the globin gene mutations defined here. HbF modulation during erythropoiesis is apparently normal in the β⁰-thal heterozygote. The proportions of Oγ and γ are similar to those seen in normal adults, and the lack of correlation in individual colonies suggests that normal adult progenitor cells were present. As expected, the only homozgyous normal family member (a 9-yr-old child) also had normal adult progenitor cells and normal regulation of HbF. Colonies from the HPFH heterozygotes also had normal adult levels of Oγ and γ-synthesis. The percent γ-synthesis did not
decrease between the BFU-E-derived colonies and reticulocytes, suggesting that deletion of the region between γβ, and 3′ to β may have a role in the normal pattern of modulation of γ-gene expression.

Patients with certain β-hemoglobinopathies (thalassemia and HPFH) thus have normal adult erythroid progenitor cells. HbF synthesis is modulated normally during erythroid development in thalassemia. This normal decrease in HbF synthesis during erythroid maturation apparently does not occur in HPFH that is associated with deletions in the β-globin gene cluster. Additional studies of hemoglobin synthesis and erythropoiesis in patients with these and other hemoglobinopathies will provide further information regarding the regulation of hemoglobin gene expression.

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Fetal hemoglobin synthesis in erythroid cultures in hereditary persistence of fetal hemoglobin and beta o-thalassemia

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