Understanding globin regulation in β-thalassemia: it's as simple as α, β, γ, δ

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A vast excess of α-globin production and inadequate γ-globin compensation lead to the development of severe anemia in human β-thalassemia. Newly identified modifiers of α- and γ-globin synthesis and insights into the mechanisms of globin regulation provide the tools for potential new approaches to treating this and other red blood cell disorders. In the study by Han and colleagues in this issue of the *JCI*, the activity of a heme-regulated protein, HRI, is shown to modulate the accumulation of excess α-globin chains in murine β-thalassemia and to decrease the severity of the disease (see the related article beginning on page 1562).

Studies of the regulation of the human β-globin gene locus have provided powerful insights into human gene expression in general at the molecular level. The human globin loci are among the best characterized in the human genome at the gene and protein levels. The β–locus control region (β-LCR) — a dominant control region located upstream of the globin structural genes — is a strong enhancer of the expression of the downstream structural globin genes (Figure 1A). The structural globin gene located furthest upstream is the ε-globin gene, which is active in early fetal life (Figure 1A). The α-globin gene and 2 γ-globin genes, Gγ and Aγ, are the major genes expressed throughout fetal life (Figure 1, B and C); the δ- and β-globin genes are activated late in fetal life, with the β-globin gene being most highly expressed in erythroid cells during adult life. Globin gene expression is controlled by the complex interactions between *cis*-acting sequences (the β-LCR and structural globin gene sequences) on the one hand and *trans*-acting factors (including transcription factors and chromatin remodeling activities) on the other. Many new details regarding these interactions have recently been described (1, 2).

The globin genes are transcribed into mRNA precursors in the nucleus and then processed to mature mRNAs, which become associated with ribosomes and are translated into globin polypeptides in the cell cytoplasm. The most stable configuration of hemoglobin is as tetramers of globin chains associated with heme groups (Figure 1C). Homozygous β-thalassemia (also known as Cooley anemia) has long been a model for the study of diseases caused by mutations and deletions at a single genetic locus, in this case, the β-globin locus. During normal fetal life, optimal γ-globin synthesis balances α-globin synthesis (Figure 1C), which results in the production of adequate amounts of fetal hemoglobin (HbF, $\alpha_2\gamma_2$). In β-thalassemia, point mutations in the β-globin structural gene are largely responsible for decreased or absent β-globin synthesis. In β-thalassemia homozygotes, γ-globin production is inadequate to compensate for the deficit in β-globin and hemoglobin A (HbA, $\alpha_2\beta_2$), despite optimal γ-globin synthesis in these patients in fetal life. As a result, a vast excess of α-globin accumulates and usually associates with heme to form hemoglobin. Possessing no single stable molecular configuration, α-hemoglobin aggregates and precipitates in early hemoglobin-producing cells in the bone marrow, which leads to apoptosis of these cells and ineffective erythropoiesis (Figure 1C). The red cells that reach the peripheral blood also contain excess α-globin; this causes the formation of inclusion bodies and an increase in reactive oxygen species levels, which leads to membrane damage and causes these cells to be preferentially hemolyzed (Figure 1C).

The current therapy for β-thalassemia is blood transfusions supplemented by iron chelation. Decreasing α-globin accumula-tion and/or reactivating γ-globin production would greatly ameliorate the anemia present in β-thalassemia. In this issue of the *JCI*, Han et al. (3) illustrate a novel mechanism for decreasing α-globin levels in a murine model of β-thalassemia. Other recent advances in understanding the fate of α-globin and the regulation of HbF synthesis have also provided new insights into the pathogenesis of human β-thalassemia and may lead to new treatments.

Decreasing excess α-globin accumulation

There are normally 2 α-globin loci located on each haploid chromosome, whose output results in normal α-globin synthesis (Figure 1, B and C). Unequal crossing over in meiosis between these α-globin loci can lead to either deletion or triplication of the α-globin gene. Deletion of α-globin loci reduces α-globin synthesis in patients homozygous for β-thalassemia, and consequently decreases the α-globin excess and the level of anemia. By contrast, the presence of extra α-globin loci results in increased α-globin accumulation and increased severity of anemia in patients with β-thalassemia.

One modifier of pathologic α-globin production in murine β-thalassemia is α-hemoglobin–stabilizing protein (AHSP), which was recently described in the *JCI* (4). AHSP binds preferentially to free α-hemoglobin, but not to β-hemoglobin or hemoglobin tetramers. AHSP-deficient ($AHSP^{-/-}$) mice have modest anemia and α-globin inclusions in their red cells (5). Kong et al. showed in their *JCI* study that $AHSP^{-/-}$ mice with β-thalassemia die in utero with a more lethal form of the disease than that of mice producing normal amounts of AHSP (4). Presumably, the binding of free α-hemoglobin by AHSP reduces pathologic α-globin precipitation by converting the free α-hemoglobin to a more nontoxic complex, perhaps by accelerating proteolysis of the excess α-globin. However, even normal levels of AHSP do not significantly prevent excess α-globin accumulation in the murine β-thalassemia model (4).
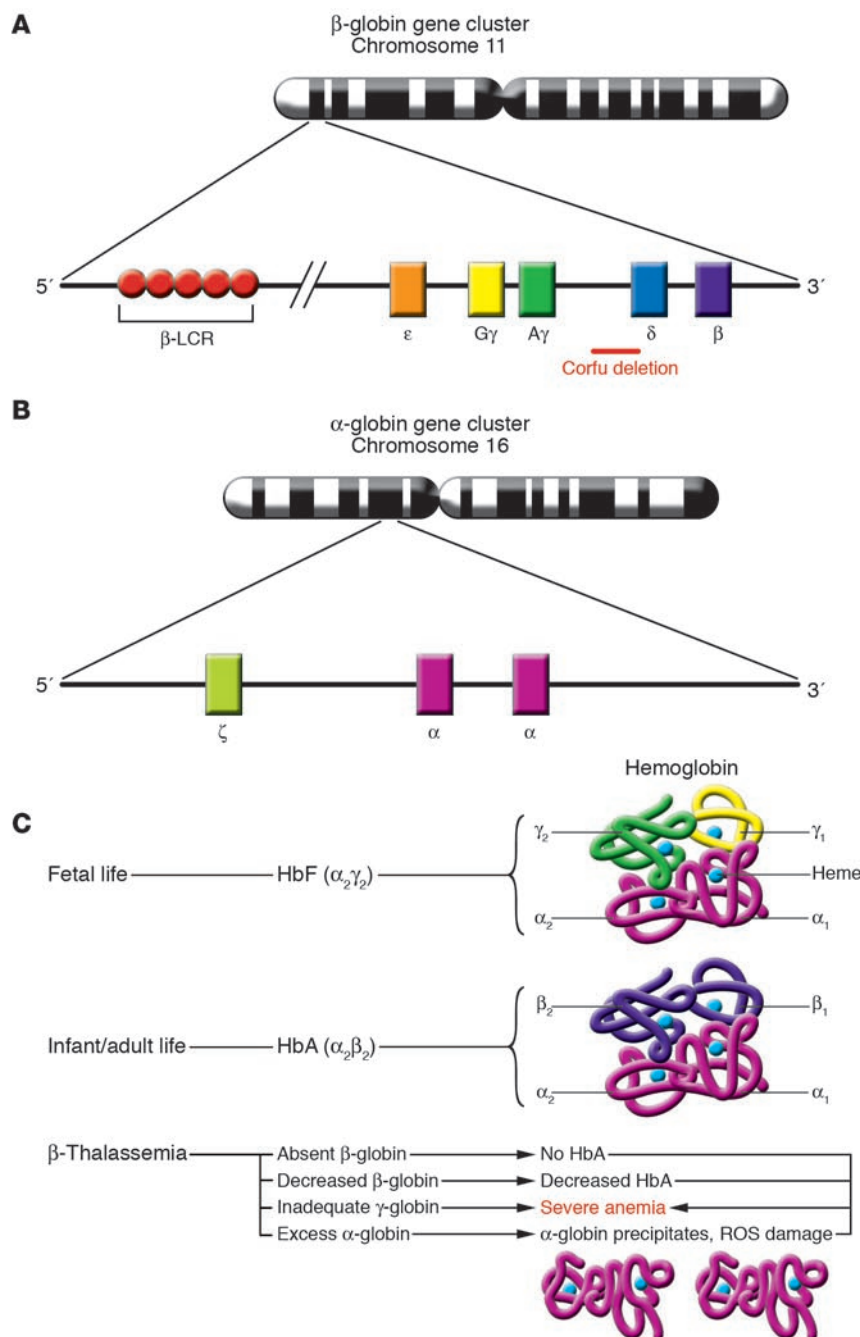
Figure 1
The human globin loci and their role in β-thalassemia. (**A**) The β-LCR and structural genes (ε, Gγ, Aγ, δ, and β) within the β-globin locus on chromosome 11 are shown. The Corfu deletion, which includes part of the structural δ-globin gene and γ–δ intergenic sequences, is also shown. (**B**) The α-globin locus is shown with the ζ- and 2 α-globin genes on chromosome 16. (**C**) In early fetal life, the α- and γ-globin chains combine to form HbF (α₂γ₂), the main β-globin–like globin during the remainder of fetal life and early postnatal life. In late postnatal and adult life, normal hemoglobin (HbA, α₂β₂) predominates. In homozygous β-thalassemia, decreased or absent β-globin production leads to decreased or absent HbA levels, respectively. The synthesis of γ-globin does not increase enough to compensate for the reduced or absent β-globin level. As a result, excess α-globin accumulates and precipitates in erythroid cells and causes damage due to the action of ROS and apoptosis of the damaged cells. Severe anemia results.

In the Han et al. article, the authors demonstrate that another gene not associated with the globin loci, heme-regulated α-subunit of eukaryotic translational initiation factor 2 (eIF2α) kinase (HRI), also reduces the severity of murine β-thalassemia by decreasing free α-globin accumulation and inclusion body formation (3). The normal role of HRI is to prevent the accumulation of α- and β-globin in the absence of heme. In cases of heme and/or iron deficiency, HRI inhibits both α- and β-globin chain translation (6). HRI-deficient ($HRI^{-/-}$) mice have increased accumulation of free α- and β-globin (6). Han et al. now show that the $HRI^{-/-}$ genotype in mice with β-thalassemia is embryonic lethal, in contrast to the less severe phenotype observed in $HRI^{+/+}$ β-thalassemic mice (3). Thus, normal HRI expression reduces the toxic effects of the vast excess of α-globin to some extent. In this same article, HRI deficiency is also shown to increase the severity of erythropoietic protoporphyria (EPP), a disease characterized by defective heme synthesis. Normal HRI activity is shown in EPP to prevent the more severe accumulation of both free α- and free β-globin chains, which form erythroid cell inclusions and are toxic to cells. This is documented in $HRI^{-/-}$ mice with EPP, in that they are more anemic than $HRI^{+/+}$ mice and have increased liver pathology and skin sensitivity (3).

Although the roles of AHSP and HRI in human disease are unknown, the fact that both decrease the severity of murine β-thalassemia suggests that strategies to reduce human α-globin excess may be useful in the treatment of human β-thalassemia. Therapeutic approaches with this goal in mind might include AHSP overexpression in human hematopoietic stem cells or the use of α-globin–specific small interfering RNAs (siRNAs) to decrease excess human α-globin accumulation.

Increasing human γ-globin expression

Another major modification at the human β-globin locus that can significantly reduce anemia and potentially cure human β-thalassemia is an increase in human γ-globin gene expression and restoration of HbF to therapeutically effective levels. Point mutations in the γ-globin gene promoter can increase γ-globin expression, but not by a great amount. By contrast, individuals with an uncommon, benign disorder known as hereditary persistence of fetal hemoglobin (HPFH) express γ-globin genes at the same level in adult life as in fetal life. Some HPFH homozygotes have only HbF and no anemia. If human β-thalassemia patients could

reactivate their HbF production to that of HPFH patients, they would be cured. The mutations associated with HPFH are large deletions at the β-globin locus extending from the region close to the human Aγ gene to well downstream of the human β-globin gene and including deletion of the structural δ- and β-globin genes (Figure 1A). The mechanism leading to the increased level of HbF in HPFH has been shown to be due, at least in part, to enhancer activity provided by the DNA sequences brought into proximity to the γ-globin genes by the deletion.

The specific role of the region between the human γ- and δ-globin genes (termed intergenic γ–δ sequences) in regulating normal hemoglobin switching and potential reactivation of HbF production in adult cells has long been postulated, but has never been clearly demonstrated in humans until an exciting recent article by Chakalova et al. (7). This report provides the first description of the hematologic findings in 2 patients homozygous for the Corfu deletion, a deletion of 7.2 kb DNA upstream of the δ-globin gene and including part of the δ-globin gene itself (Figure 1A) (7). The 2 Corfu homozygotes were shown to possess 88% and 90% HbF and only mild anemia and did not require blood transfusions, reminiscent of HPFH patients. To my knowledge, these data provide the first strong evidence in humans that intergenic γ–δ sequences are important in γ-globin gene regulation. They also show that near-complete reactivation of the human γ-globin gene in adult-type human erythroid cells can occur as a result of the Corfu deletion alone and that the deletion can reverse human γ-globin "silencing" (7). These results also suggest that intergenic γ–δ sequences within the Corfu deletion may also play a role in normal human γ-to-β globin switching in late fetal life.

The mechanisms by which the Corfu deletion of γ–δ intergenic sequences upregulate γ-globin and HbF expression remain to be determined. One model for this activity is that chromatin remodeling complexes that are developmental stage specific might act by changing the conformation of chromatin in the γ–δ region and thus modifying the interactions between the β-LCR and the downstream globin structural genes (Figure 2) (8). Our group has described such a chromatin remodeling complex, the polypyrimidine (PYR) complex, so named because of its PYR-rich DNA-binding site 1 kb upstream of the human δ-globin gene and located within the Corfu deletion (Figure 1) (8, 9). PYR complex is adult

hematopoietic cell specific, because the transcription factor Ikaros required for PYR complex formation is primarily expressed in adult hematopoietic cells (Figure 2) (8, 10). Ikaros-null mice, which lack Ikaros protein expression, have no PYR complex and have delayed mouse and human globin switching (10). PYR complex contains subunits of 2 chromatin remodeling complexes, one known to activate gene transcription and another that represses gene expression and includes histone deacetylases (HDACs) as subunits (9, 11).

Taken together, the new data from the Corfu patients and PYR complex suggest a model in which PYR complex functions in the human intergenic γ–δ sequences as

a γ-to-β–switch complex by remodeling chromatin and repressing γ-globin gene expression in adult-type hematopoietic and erythroid cells (Figure 2). The Corfu deletion may work, at least in part, by preventing PYR complex binding in adult hematopoietic cells and thus permitting human γ-globin reactivation.

Butyrate compounds are known to increase HbF levels in adult-type erythroid cells in patients with sickle cell disease and β-thalassemia. Butyrate is also known to inhibit HDACs and thus may work by interfering with PYR complex action and therefore de-repressing the human γ-globin genes (12, 13). It is probable that other chromatin-remodeling complexes associ-
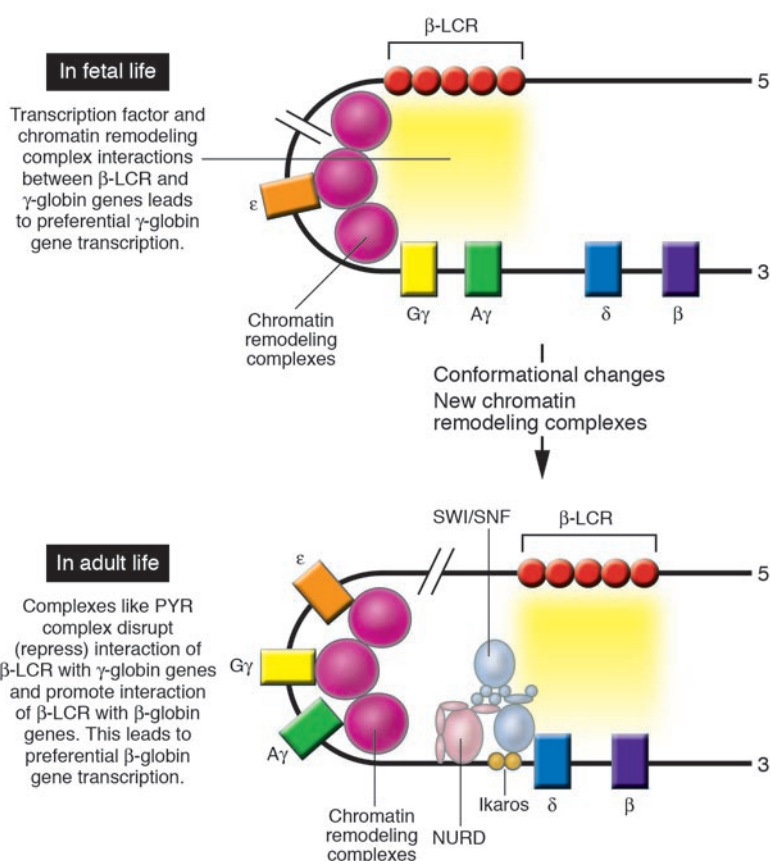

Figure 2
A model for human γ-to-β globin switching and γ-globin reactivation in adult hematopoietic cells. In fetal life, the human γ-globin genes are preferentially activated by interactions between the β-LCR and the γ-globin genes mediated by transcription factors and chromatin remodeling complexes. The details of these complexes at this time are unknown. In adult-type hematopoietic cells, chromatin remodeling complexes repress γ-globin transcription, change the conformation of the β-globin locus by binding to the intergenic γ–δ sequences, and favor interactions between the β-LCR and the downstream β-globin gene. PYR complex, shown here binding to the region upstream of the human δ-globin gene (and included in the Corfu deletion) with Ikaros as its DNA-binding subunit, is a known adult erythroid stage–specific chromatin remodeling complex that may function in this process. The subunits of PYR complex are shown. These include SWI/SNF subunits, parts of an ATP-generated chromatin remodeling complex that activate gene transcription; NURD subunits, parts of a repressive chromatin remodeling complex with HDACs; and Ikaros as the DNA-binding subunit that holds the complex together (11).

ated with other erythroid transcription factors binding in the intergenic γ–δ sequences are also active in human globin switching in both fetal and adult-type hematopoietic progenitors and erythroid cells. Inhibition of the activity of these complexes — for example, by using siRNAs directed against Ikaros or other components of other complexes — is a potential approach to reactivating human γ-globin expression.

The data in the Chakalova et al. article also indicate an inverse relationship between γ- and β-globin accumulation in adult erythroid cells (7). When there is more β-globin and HbA present, as found in the cells of patients doubly heterozygous for Corfu and β-thalassemia genes and patients heterozygous for the Corfu deletion, there are lower-than-expected levels of γ-globin and HbF accumulation (7). These results suggest that in patients treated with butyrate or other HbF-inducing agents, downregulating human β-globin expression may be a useful approach to further optimize HbF production.

In summary, upregulation of γ-globin and/or downregulation of α-globin could reestablish normal globin balance between

α- and non–α-globin chains and avoid the excess α-globin toxicity primarily responsible for anemia in human β-thalassemia. Achieving such a balance could ameliorate or even cure patients with these diseases. New insights into the mechanisms of globin regulation, such as those of Han et al. (3), may eventually lead to new, more rational treatments for patients with β-thalassemia.

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1. Patrinos, G.P., et al. 2004. Multiple interactions between regulatory regions are required to stabilize an active chromatin hub. *Genes Dev.* **18**:1495–1509.
2. Vakoc, C.R., et al. 2005. Proximity among distant regulatory elements at the β-globin locus requires GATA-1 and FOG-1. *Mol. Cell.* **17**:453–462.
3. Han, A.-P., Fleming, M.D., and Chen, J.-J. 2005. Heme-regulated eIF2α kinase modifies the phenotypic severity of murine models of erythropoietic protoporphyria and β-thalassemia. *J. Clin. Invest.* **115**:1562–1570. doi:10.1172/JCI24141.
4. Kong, Y., et al. 2004. Loss of α-hemoglobin–stabilizing protein impairs erythropoiesis and exacerbates β-thalassemia. *J. Clin. Invest.* **114**:1457–1466. doi:10.1172/JCI200421982.
5. Kihm, A.J., et al. 2002. An abundant erythroid protein that stabilizes free α-haemoglobin. *Nature.* **417**:758–763.
6. Han, A.P., et al. 2001. Heme-regulated eIF2α kinase (HRI) is required for translational regulation and survival of erythroid precursors in iron deficiency. *EMBO J.* **20**:6909–6918.
7. Chakalova, L., et al. 2005. The Corfu δβ thalassemia deletion disrupts γ-globin gene silencing and reveals post-transcriptional regulation of HbF expression. *Blood.* **105**:2154–2160.
8. O'Neill, D., Bornschlegel, K., Flamm, M., Castle, M., and Bank, A. 1991. A DNA-binding factor in adult hematopoietic cells interacts with a pyrimidine-rich domain upstream from the human δ-globin gene. *Proc. Natl. Acad. Sci. U. S. A.* **88**:8953–8957.
9. O'Neill, D., et al. 1999. Tissue-specific and developmental stage-specific binding of a SWI/SNF complex associated with human fetal to adult globin switching. *Proc. Natl. Acad. Sci. U. S. A.* **96**:349–354.
10. Lopez, R.A., Schoetz, S., DeAngelis, K., O'Neill, D., and Bank, A. 2002. Multiple hematopoietic defects and delayed globin switching in Ikaros null mice. *Proc. Natl. Acad. Sci. U. S. A.* **99**:602–607.
11. O'Neill, D., et al. 2000. An Ikaros-containing chromatin-remodeling complex in adult-type erythroid cells. *Mol. Cell. Biol.* **20**:7572–7582.
12. Chen, J.S., Faller, D.V., and Spanjaard, R.A. 2003. Short-chain fatty acid inhibitors of histone deacetylases: promising anticancer therapeutics? *Curr. Cancer Drug Targets.* **3**:219–236.
13. Davie, J.R. 2003. Inhibition of histone deacetylase activity by butyrate. *J. Nutr.* **133**:2485S–2493S.

An oily, sustained counter-regulatory response to TB

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Lipoxins are potent antiinflammatory lipid mediators that restrain and promote the resolution of a wide variety of inflammatory processes. Recent studies implicating deficient lipoxin production in the pathogenesis of diverse inflammatory diseases, along with numerous reports of the beneficial effects of lipoxin analog administration in animal models of inflammatory pathology, have suggested that harnessing the pleiotropic activities of the lipoxins is a strategy with considerable therapeutic promise. In this issue of the *JCI*, Bafica et al. address the other side of the coin, reporting that endogenous lipoxins compromise immune-mediated control of *Mycobacterium tuberculosis* infection in mice (see the related article beginning on page 1601). In addition to providing novel insight into the mechanisms that interfere with the development of protective immune responses to *M. tuberculosis*, the study raises the possibility that pharmacological inhibition of lipoxin synthesis may provide a method of augmenting inefficient immune responses in TB and other important chronic infectious diseases.

Maintenance of health is critically dependent upon the immune system's ability to generate a balanced response to a variety of threats, real or perceived. Inflammatory responses of insufficient vigor can allow uncontrolled pathogen replication, events

central to the development of malaria, TB, and HIV, the top infectious killers in the world today. On the other hand, excessive or inappropriate inflammatory responses place an equally heavy burden on humanity, being key to the pathogenesis of diverse infectious (e.g., sepsis, fulminant viral hepatitis), autoimmune (e.g., inflammatory bowel disease, multiple sclerosis), allergic (e.g., asthma), genetic (e.g., cystic fibrosis), and degenerative (e.g., atherosclerosis) diseases. It is thus not surprising that there has been considerable experimental, theoretical, and therapeutic interest in the molecular mechanisms that restrain the intensity of inflammatory responses. In addition to the usual suspects, such as cytokines, receptors, intracellular signaling inhibitors, and specialized suppressor cells, endogenous antiinflammatory lipid mediators have recently been recognized as playing an important role.