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 locus located furthest upstream is the ε-globin gene, which is active in early fetal life (Figure 1A). The α-globin locus and 2 γ-globin genes, Gγ1 and Gγ2, are the major genes expressed throughout fetal life (Figure 1A) 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 α-globin synthesis balances α-globin synthesis (Figure 1C), which results in the production of adequate amounts of fetal hemoglobin (HbF, α2γ2). 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, α2β2), 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 accumulation 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 1A, 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).

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

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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 developmentally 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-
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.

Nonstandard abbreviations used: LO, lipoxigenase; LXA₄, lipoxin A₄; NOS2, NO synthase 2.

Conflict of interest: The authors have declared that no conflict of interest exists.