blood commentary

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Miyata et al, page 269

A Cambrian origin for globin gene regulation

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In this issue of *Blood*, Miyata et al report on their studies in lampreys, a vertebrate far distant from mammals, and infer that a core component of globin gene regulation became active long ago, likely in the Cambrian Period (about 430 to 540 million years ago).[1]

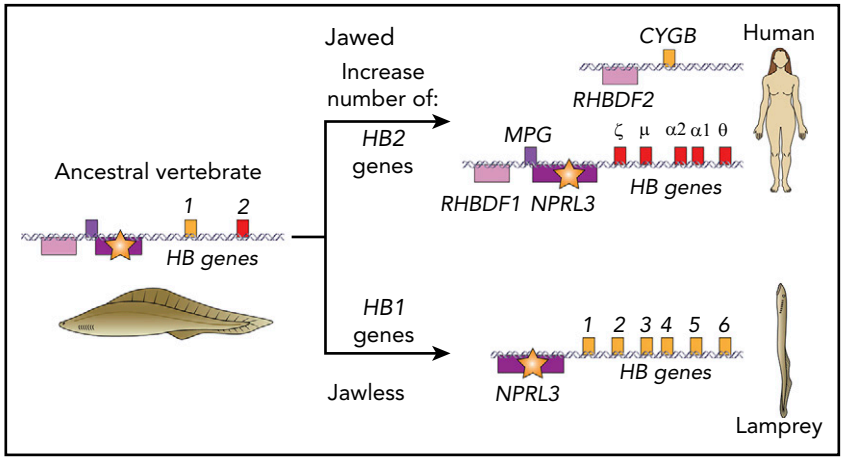
Comparisons of hemoglobin gene loci across a broad range of mammalian species have revealed much about both their evolutionary histories and their regulation (eg, locations of many *cis*-regulatory elements were predicted by strong noncoding sequence conservation). However, equivalent studies in vertebrate species more distant from humans and mice are more challenging, mainly because regulatory element sequences evolve rapidly compared with the slower rate of evolution of protein-coding sequences. The exons of homologous protein-coding genes can align well in comparisons of genomic sequences from humans to invertebrates or beyond, but human regulatory regions rarely align with sequences more distant than marsupials.[2] Thus, comparative genomic analyses relevant to the regulation of human genes can be applied across mammalian species (perhaps out to 160 million years ago), but the challenges for examining longer histories are formidable.

One question requiring a long evolutionary view is the origin of regulatory elements that direct the high-level production of the globin polypeptides of hemoglobins (Hgb's, encoded by *HB* genes) in vertebrate erythroid cells. Efforts to manipulate the sequences of such regulatory elements, or proteins acting at them, are at the heart of multiple efforts to redirect globin expression for therapeutic purposes, such as reactivation of fetal Hgb in adults with sickle cell disease or other hemoglobinopathies. This keen interest in globin gene regulatory elements has driven decades of research that has revealed that the introns of the gene *NPRL3* are needed for high-level expression of the genes encoding the α-globin subunits of Hgb in humans and mice[3] (see figure). An intron of the homologous *NPRL3* gene in zebrafish also enhances expression of the linked *HB* genes.[4] Notably, the intron in fish *NPRL3* shares a common function (enhancement) with equivalent introns in mammals, despite a lack of sequence similarity.[5]

Miyata et al extended our view of globin regulatory elements much further back in history. They examined the lamprey, a member of the vertebrate clade most distant from mammals. About 500 million years ago, ancestral vertebrates separated into 2 major groups, the jawed vertebrates (leading to the evolution of mammals, birds, and fish) and the jawless vertebrates (leading to the evolution of hagfish and lampreys). The Hgb's in both jawed and jawless vertebrates transport oxygen within erythrocytes, but surprisingly, the globin genes in the jawless species are more similar to the *CYGB* gene encoding cytoglobin,[6] a protein widely distributed across cell types in most vertebrates that usually does not have a role in oxygen transport.[7] Thus, it seems that the oxygen-transporting activity of *HB* genes arose by convergent evolution in the 2 branches of vertebrates, from a *CYGB*-like ancestral gene in jawless vertebrates and from an ancestral gene related to canonical *HB* genes (eg, *HBA* and *HBB* genes in mammals) in jawed vertebrates.

If the oxygen-transporting function of Hgb's arose twice in vertebrates, did the elements responsible for high-level expression in erythroid cells also arise twice? Miyata et al tackled this difficult question with a mix of genomics, biochemistry, and genetics. After assembling a DNA sequence of the globin gene locus from the river lamprey (*Lampetra fluviatilis*), the authors discovered 6 *HB* genes with an *NPRL3* gene just upstream (see figure). The arrangement and orientations of the genes were similar to those in humans, raising the intriguing possibility that the introns of lamprey *NRPL3* could harbor a critical regulatory element. The authors searched the locus for a biochemical signature common to most regulatory elements, accessibility of chromatin, and they found an accessible chromatin site in intron 7 of *NRPL3* in lamprey erythrocytes. They then tested the function genetically, using transient transgenesis in lampreys to show that intron 7 of lamprey *NPRL3* could drive erythroid-specific expression of a reporter gene. Importantly, lamprey intron 7 was not active in transgenic assays in more conventional model species such as zebrafish, showing the need for conducting functional analyses in an appropriate context, in this case transgenesis in a closely related species, the sea lamprey. Thus, an intronic enhancer in *NPRL3* is regulating the expression of linked *HB* genes in erythroid cells of both major clades of vertebrates, despite the fact that the genes in those *HB* clusters are derived from different ancestral genes.

These results led to a model (see figure) to explain the conundrum of convergent evolution of protein-coding genes while sharing an ancestral regulatory element. More than 500 million years ago, during the enormous diversification of animals in the seas of the Cambrian Period, an



A globin gene regulatory element discovered in lampreys suggests an ancient origin in ancestral vertebrates. The Miyata et al study of lampreys showed that genes encoding globin polypeptides of the oxygen transporter hemoglobin (*HB* genes, light orange and red boxes) are adjacent to a ubiquitously expressed *NPRL3* gene (violet box) in both major branches of vertebrates, jawed and jawless, despite the separate, convergent evolution of *HB* genes in each branch, with *HB* genes in jawless vertebrates more related to *CYGB* (light orange boxes). Furthermore, an intron of lamprey *NPRL3* contains a major regulatory element for globin genes (star), as is the situation in humans. These maps in extant species suggest that the linkage of *NPRL3*, containing a strong regulatory element, to *HB* genes occurred in an ancestral vertebrate, represented as *Haikouichthys ercaicunensis*.[10] By hypothesizing multiple ancestral *HB* genes in the linkage group, one related to *CYGB* and another related to canonical vertebrate *HB* (red box), the model can explain convergent evolution of different oxygen-transporting globins as selective expansions of one or the other gene while maintaining strong regulation from the *NPRL3* intronic enhancer. Additional genes characteristic of this locus are also shown; boxes above the illustrative DNA helices are transcribed left to right, and those below the DNA are transcribed right to left. The *CYBG* gene and *HB* genes are on different chromosomes in humans.

NPRL3 gene with a strong regulatory element became linked to at least 2 different globin genes in the ancestor to vertebrates. Hints from gene arrangements in tunicates suggest that *NPRL3* was not linked to globin genes before this time. That strong regulatory element remained active, leading to high-level expression of the linked *HB* genes in both clades of vertebrates. However, in adapting to the need for oxygen transport, the ancestral globin gene corresponding to canonical hemoglobin genes expanded and diversified in jawed vertebrates, whereas the ancestral globin gene corresponding to *CYGB* had a similar fate in jawless vertebrates.

The new results show that gene regulatory elements can be deeply preserved over evolutionary time, long past the time frame illuminated by comparative genomics, but only careful biochemical and genetic analyses will reveal them. However, it is important to keep in mind that many regulatory elements are not strongly conserved; perhaps a majority of regulatory elements arose recently within specific lineages or have been repurposed for new functions.[8,9] The authors deduce a model that helps resolve a previously difficult evolutionary scenario. Still, many questions remain, including how the globin gene clusters arose within each clade and how various globins have been adapted to different functions across clades. It is likely that comparative biochemical and functional analyses of globin genes will remain fruitful for many future studies.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Holstein et al, page 279

Bleeding in acquired hemophilia: have we figured it out?

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In this issue of *Blood*, Holstein et al show the extent to which the risk of bleeding after a diagnosis of acquired hemophilia A (AHA) remains significant until near-normal factor VIII (FVIII) level is attained.[1]

All consultant hematologists, whether from the benign or malignant side of our specialty, have a duty to recognize the cardinal signs of AHA, a rare but severe autoimmune condition where diagnosis is frequently and dangerously delayed.[2] Cardinal signs are unexplained excessive bleeding, usually in an elderly person