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Advances in hemoglobin disorders - Section 1

Switching genes on and off during erythropoiesis

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Take Home Messages

- Erythropoiesis provides an outstanding model of lineage specification, differentiation and terminal maturation relevant to all such systems.
- Understanding regulation of the α-globin cluster illustrates many of the principles underlying mammalian gene expression.
- Understanding how the globin genes are switched on and off during erythropoiesis offers new opportunities to modify these switches in human genetic disease.

Introduction

A fundamental question in current biology is to understand how genes are switched on and off during cell fate decisions. The hematopoietic system provides the ideal system in which to investigate this question, largely because it is possible to isolate and analyze populations of cells at specific stages of lineage commitment and differentiation, as multipotent hematopoietic stem cells produce progenitors, defined precursors and ultimately mature blood cells. This process can be studied in embryonic, fetal and adult life providing additional information on developmental control of gene expression. In this context, the detailed mechanisms by which mammalian genes are regulated have often been elucidated by studying the α- and β-like globin genes, which are silent in early progenitors but expressed at increasingly high levels throughout terminal maturation, soon after commitment to the erythroid lineage. In addition, studying disorders of globin expression (e.g. thalassemia and sickle cell disease) has illustrated many of the general principles by which mutations can give rise to human genetic diseases. Analysis of the globin genes has also pioneered the application of genetic engineering to human diseases, being amongst the first examples of successful gene therapy, and providing ideal targets for current developments in therapeutic genome editing.

Current state-of-the-art

We study the mechanisms by which mammalian genes are switched on and off by analyzing how the human and mouse α-globin gene clusters are regulated during erythropoiesis (see figure). Past experience has shown that principles established using this model apply to a large proportion of other mammalian genes. Although there are some differences in how humans and mice control globin gene expression, there are many similarities making the mouse a good experimental model for understanding the mechanisms involved. We have previously defined a ~120kb segment of the human genome that contains all of the information required to produce normal tissue- and developmental-stage specific expression of the α-globin cluster.[1] This corresponds to a homologous, syntenic region of ~70kb in the mouse. This minimal regulatory region broadly corresponds to recently described self-interacting domains of chromatin (referred to as topologically associated domains: TADs).[2]

Next, we have addressed which specific sequences within the α-globin TAD are required to produce fully regulated α-globin expression. As for other cell-specific genes we have found that α-globin expression is controlled by three overlapping classes of *cis*-acting elements; promoters, enhancers and boundary elements. Promoters are found at regions corresponding to the transcription start sites of genes. By contrast, enhancers are scattered in distal regions around the genes whose expression they regulate. Regulatory domains and TADs are surrounded by boundary elements. The distribution of these elements at the human α-globin cluster are shown in the figure.

Most promoters contain binding sites for widely-expressed transcription factors (TFs) and elements which recruit the so-called pre-initiation multi-protein complex (PIC). When assembled, this complex recruits RNA Polymerase II (Pol II), which transcribes the gene in question. By contrast most enhancers bind a combination of broadly expressed TFs and co-factors but importantly they bind cell-specific TFs which are important for establishing the identity of each specific blood lineage. In the case of erythropoiesis, the key TFs are GATA1, the SCL complex, NFE-2 and KLF1 (Figure 1).[3] Enhancers become activated when they are bound by these factors and they physically interact with their target promoter, which may lie 10s-1000s kb away, and increase expression of target genes which encode the characteristic proteins that make specific cell types. In the case of one such target gene (α-globin) there are four enhancers (R1-R4) lying 10, 33, 40 and 48kb upstream of the ζ-promoter respectively (Figure 1). More recently researchers have been analyzing the role of boundary elements. These are small regions of the genome that bind an architectural protein called CCCTC-binding factor (CTCF), and a protein involved in the structural maintenance of

chromosomes called cohesin.[4] As for many other loci, in the globin clusters such elements flank the TAD containing the genes, their promoters and the enhancers. Currently it is thought that one important role of boundary elements is to confine the enhancers to work within such defined regions of the genome, preventing inappropriate activation of nearby genes by the enhancers.[2]

The ability to purify cells representing all stages of erythropoiesis from the earliest committed erythroid cells (called burst forming units BFU-e) through to the enucleated red cell has allowed us to study the dynamics of globin gene expression. We can thus study changes at the α-globin locus as the transcriptional program (expression and binding of TFs) and the associated epigenetic changes (e.g. nuclear position, chromatin modifications, DNA methylation) evolve as the globin genes are switched on and off during erythropoiesis. Surprisingly many of the key TFs and active epigenetic modifications appear to be in place before the globin genes are expressed.[5] Furthermore, we have found that the α-globin enhancers appear to increase expression of the α-globin genes by influencing the recruitment of Pol II.[6]

Future perspectives

Further understanding of what is already a well-characterized picture of α-globin gene expression will continue to provide a pre-eminent model of mammalian gene expression, the framework for understanding the mechanisms underlying human genetic disease. However, our current understanding also suggests a possible therapeutic approach in patients with some forms of β-thalassemia. The pathophysiology of β-thalassemia can be explained by the excess of α-globin chains that precipitate in erythroid precursors and red blood cells, producing both dyserythropoiesis and hemolysis. We have recently shown that using programmable (CRISPR-Cas9) nucleases, it is possible to redress globin chain imbalance by down-regulating α-globin expression in the HSCs of patients with β-thalassemia by deleting a key enhancer (R2 in the figure). Although there are many issues to be addressed to modify the HSCs of patients with β-thalassemia, it seems that isolating HSCs, modifying the α-globin enhancer and re-introducing modified cells in an autologous stem cell transplant may be a feasible approach to ameliorate the hematological and clinical phenotypes of affected individuals.[7]

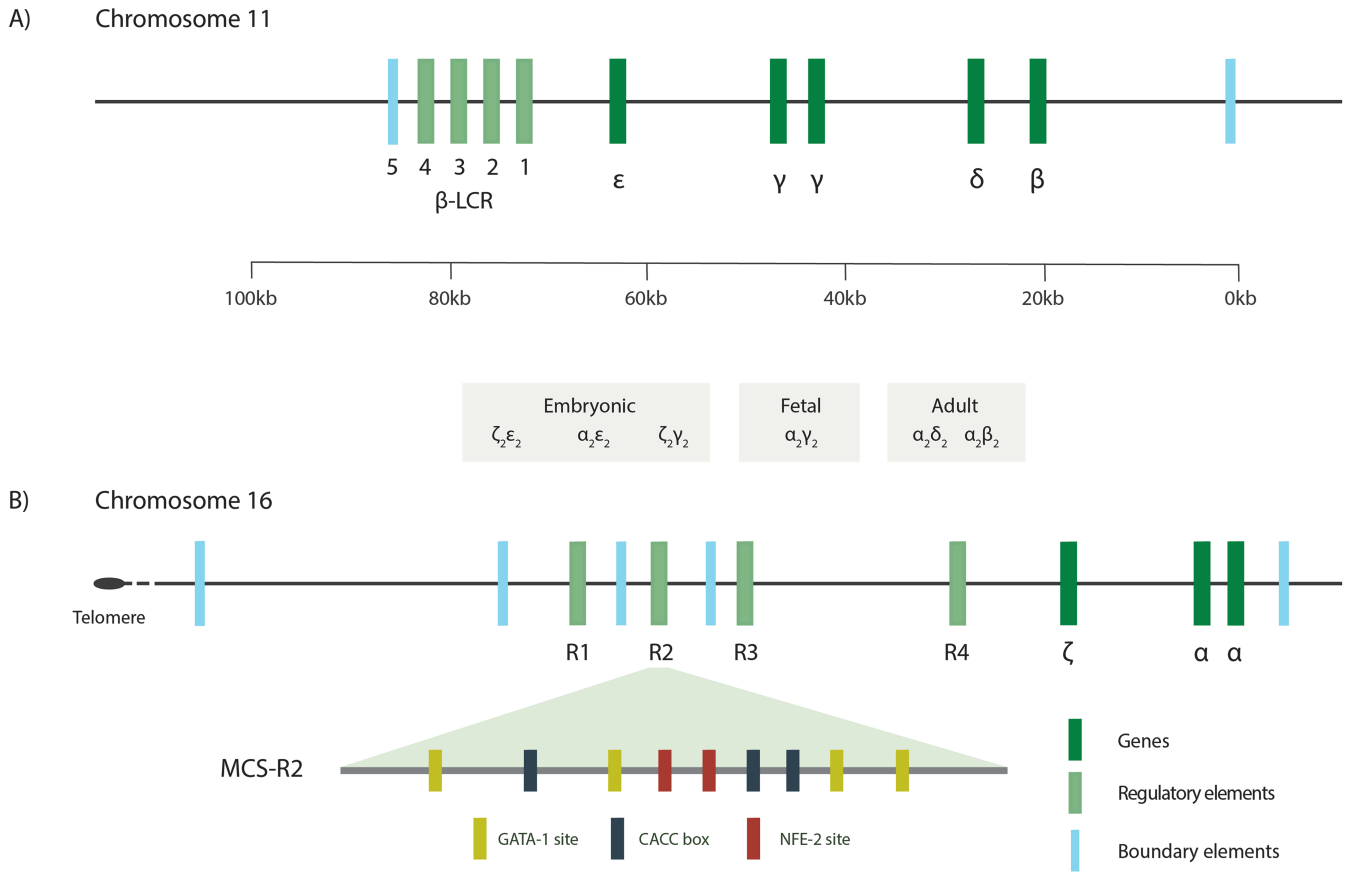

Figure 1. A schematic diagram of the human α- and β-globin gene clusters with regulatory elements, and the developmental stage-specific hemoglobin molecules produced. Genes are arranged at each locus in a syntenic manner, in the order in which they are expressed during development. Several boundary elements are situated at each cluster. A) At the β cluster ε- (embryonic), γ- (fetal and adult), δ- (adult) and β-globin (adult) are produced. The regulatory elements 1-5 are situated at the β-locus control region B) The α-cluster lies in the sub-telomeric region of chromosome 16. ζ- (embryonic) and then α-globin (adult) are produced, and the regulatory elements are known as MCS-R1-4 respectively. The main element, MCS-R2 is well conserved through mammalian evolution and contains several key erythroid transcription-factor binding sites. The hemoglobin types expressed during different stages of development are embryonic: Hb Gower-I ($\zeta_2\varepsilon_2$), Hb Gower-II (a_2e_2), and Hb Portland ($\zeta_2\gamma_2$), fetal: HbF ($\alpha_2\gamma_2$), and adult: HbA ($\alpha_2\beta_2$), and HbA_2 ($\alpha_2\delta_2$).

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