Physiological and Aberrant γ-Globin Transcription During Development

Gloria Barbarani†, Agata Labedz†, Sarah Stucchi, Alessia Abbiati and Antonella E. Ronchi*

Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Milano, Italy

The expression of the fetal Gγ- and Aγ-globin genes in normal development is confined to the fetal period, where two γ-globin chains assemble with two α-globin chains to form α2γ2 tetramers (HbF). HbF sustains oxygen delivery to tissues until birth, when β-globin replaces γ-globin, leading to the formation of α2β2 tetramers (HbA). However, in different benign and pathological conditions, HbF is expressed in adult cells, as it happens in the hereditary persistence of fetal hemoglobin, in anemias and in some leukemias. The molecular basis of γ-globin differential expression in the fetus and of its inappropriate activation in adult cells is largely unknown, although in recent years, a few transcription factors involved in this process have been identified. The recent discovery that fetal cells can persist to adulthood and contribute to disease raises the possibility that postnatal γ-globin expression could, in some cases, represent the signature of the fetal cellular origin.

Keywords: globin genes, transcription factors, hereditary persistence of fetal hemoglobin, juvenile myelomonocytic leukemia, erythropoiesis

ERYTHROPOIESIS DURING DEVELOPMENT

During mammalian development, hematopoiesis is regulated both spatially and temporally: it begins in the yolk sac, it goes through a transitory phase in the fetal liver and then is definitively established in the thymus and bone marrow (Dzierzak and Bigas, 2018). The first erythroid precursors emerge from the yolk sac as soon as the embryo grows too big to be supplied with oxygen by diffusion and give rise to primitive erythroid cells (EryPs). These cells are released in the bloodstream when they are still nucleated and are characterized by the expression of embryonic globins. A second wave of cells migrating into the fetal liver from the yolk sac support fetal hematopoiesis until birth, in the interval between primitive and definitive hematopoietic stem cell (HSC)-dependent hematopoiesis. Of interest, recent studies suggest that in mouse, this second fetal transient hematopoietic wave of yolk sac-derived erythro-myeloid progenitors (EMPs) may persist postnatally (Epelman et al., 2014; Gomez Perdigueró et al., 2015). HSCs arise from the hemogenic endothelium of the embryonic aorta-gonad-mesonephros (AGM), the vitelline and umbilical arteries, and from the placenta (Dzierzak and Philipsen, 2013; Lacaud and Kouskoff, 2017; Dzierzak and Bigas, 2018). These cells migrate first into the FL and then to the bone marrow (BM)—their long-term adult resident location—where they will last throughout life and will generate all types of blood cells, including erythrocytes (Orkin and Zon, 2008; Palis, 2014; Dzierzak and Bigas, 2018).

The different types of erythroid cells produced at the different hematopoietic stages have many common characteristics, including the main steps of progressive differentiation and maturation.
from early progenitors to erythroblasts and finally to red blood cells (RBCs) (Kina et al., 2000; Aisen, 2004; Chen et al., 2009; Baron, 2013; Palis, 2014). However, importantly, they can be in part distinguished by differences in cell morphology and in the expression of embryo/fetal vs. adult globins.

GLOBIN GENES

In humans, the α-globin cluster contains three functional genes: the embryonic, HBZ (ζ-globin) and the two fetal/adult HBA2 and HBA1 duplicated genes (α2- and α1-globin) (Stamatoyannopoulos, 2005). The β-globin cluster contains five active genes: the embryonic HBE (ε-globin) gene, the two highly homologous fetal HBG2 and HBG1 genes (γy- and Ay-globin, respectively) and the two adult HBD and HBB genes (δ- and β-globin, the latter accounting for about 98% of adult β-like globin) (Figure 1). Each locus is under the control of a set of distal enhancers (Grosveld et al., 1987; Higgs et al., 1990). The genes contained in the α-globin and β-globin loci are sequentially expressed in a stage-specific manner that maintains a 1:1 ratio between the α-like and β-like globin chains, in a process known as "hemoglobin switching" (Forget, 1990; Stamatoyannopoulos, 2005; Sankaran et al., 2010a).

Interestingly, the presence of fetus-specific (γ) genes and thus of a fetal (γ) to adult (β) globin switch is unique to humans and old-world monkeys: most species, including mice, have only one switch, from embryo/fetal to definitive globin genes expression, occurring early in development (Stamatoyannopoulos, 1991; Sankaran et al., 2010a; Philipson and Hardison, 2018; Figure 1). In mice transgenic for the human β-globin locus, the switching of human globin genes parallels the switching of mouse genes, with γ genes being switched off between E11.5 and E13.5, together with ey and bH1 mouse embryo/fetal genes (Stroubloulis et al., 1992; Peterson et al., 1995). Each developmental switch is accompanied by a profound chromatin remodeling within the loci: the interaction ("loop") between the promoter of the gene active at a given time with the common distal enhancers [locus control region (LCR)] is progressively favored, with inactive globin genes being looped out (Tolhuis et al., 2002; Palstra et al., 2003). Whereas only EryPs of yolk sac origin expresses embryonic ζ-, human ε- and mouse ζ-globin genes, the other globin genes are more promiscuously expressed by cells of different origin. McGrath et al. (2011) showed that the first cells expressing adult globins, prior to the generation of HSC-derived erythroblasts, are indeed the transient population of EMP-derived erythroid cells.

PHYSIOLOGICAL AND NON PHYSIOLOGICAL γ-GLOBIN EXPRESSION

The switching from γ- to β-globin expression is the most intensively studied because the persistence of γ-globin expression in adult stages is a hallmark of a very heterogeneous spectrum of conditions. These can be benign, as in the case of the few F cells (cells expressing HbF) found in normal adults (Boyer et al., 1975) and in HPFH (hereditary persistence of fetal hemoglobin) (Forget, 1998), or associated with disease, such as in response to transient or chronic anemias (Weatherall, 2001) or leukemias (Weatherall et al., 1968; Sheridan et al., 1976). As an additional reason of interest, the ability of reactivating γ-globin in the adult is considered as a possible strategy to cure β-hemoglobinopathies (Wienert et al., 2018). The cause of γ-globin expression in adult cells remains largely unknown and is thought to rely on different mechanisms, both maturational and/or directly related to defects intrinsic to the HBB locus (Zago et al., 1979; Stamatoyannopoulos, 2005). Although these aspects are strictly intertwined, and it is almost impossible to sharply separate them, this review will focus on the latter, in particular on the major transcription factors (TFs) that, by directly binding to the HBB locus, act as selective on/off switches of γ-globin expression in normal and aberrant conditions.

THE TRANSCRIPTIONAL SWITCHES OF γ-GLOBIN EXPRESSION

The observation that the main differentiation and maturational steps leading to RBC formation are common to YS-, EMP-, and HSC-derived erythroblasts (Kina et al., 2000; Aisen, 2004; Chen et al., 2009; Palis, 2014) suggests that these cells may also rely on a common set of transcription factors directing erythroid differentiation and globin gene regulation. Indeed, embryonic/fetal and adult cells share a common set of ubiquitous (such as NF-Y, that binds all globin promoters, although with different affinity) (Liberati et al., 1998; Zhu et al., 2012; Martyn et al., 2017)) and erythroid-specific activators/coactivators [first of all GATA1 (Ferreira et al., 2005; Love et al., 2014; Katsumura et al., 2017); Barbarani et al., 2019], NFE2(Gasiorek and Blank, 2015; Kim et al., 2016), KLF1(Perkins et al., 2016), and TAL1 (Kang et al., 2015)]. Moreover, the expression of γ-globin genes in adult cells, as in HPFH, suggests that adult cells represent an environment permissive for the expression of both embryo/fetal and adult globin genes. It is thus likely that the specific timing of γ-globin expression might require specific activators/repressors acting at different times. The number of transcription factors directly involved in the activation/repression of γ-globin transcription is surprisingly small, and their main characteristics are briefly reviewed here below (Figure 2).

TRANSCRIPTION FACTORS AFFECTING THE EMBRYO/FETAL TO ADULT SWITCHING

γ-Globin Repressors

BCL11A, also known as CTIP1 (Coup-TFI interacting protein), is a C2H2-type zinc-finger protein (Avram et al., 2000). Alternative splicing generates four major protein isoforms, sharing a common N-terminus: eXtra-Long (XL), Long (L), Short (S), and eXtra-Short (XS) (Liu et al., 2006). BCL11A plays important roles in non-erythroid hematopoietic cells,
reactivate targeted inactivation in erythroid cells a promising approach to (Xu et al., 2013). This latter observation made BCL11A- (Xu et al., 2015) impairs HbF silencing in adult erythroid cells, without specific BCL11A enhancer (Bauer et al., 2013; Canver et al., 2018) obtained by disrupting the erythroid-compartment (Tsang et al., 2015; Greig et al., 2016) and possibly for the differentiation of other lineages, such as breast (Khaled et al., 2015) and pancreas (Peiris et al., 2018) cells. Its specific role in γ-globin silencing was identified by genome-wide association studies (GWAS) aiming to identify eQTLs (expression quantitative trait loci) associated with high levels of postnatal HbF (Menzel et al., 2007; Lettre et al., 2008; Uda et al., 2008). Its conditional knockout within the erythroid compartment [obtained by disrupting the erythroid-specific BCL11A enhancer (Bauer et al., 2013; Canver et al., 2015)] impairs HbF silencing in adult erythroid cells, without altering erythropoiesis in the mouse (Sankaran et al., 2008, 2010b; Xu et al., 2013). This latter observation made BCL11A-targeted inactivation in erythroid cells a promising approach to reactivate γ-globin in β-hemoglobinopathies (Sankaran et al., 2010a; Wienert et al., 2018; Zeng et al., 2020). Interestingly, in human cells, S and XS isoforms are specific of YS primitive and FL erythropoiesis, whereas XL and L are specific of BM definitive erythropoiesis (Sankaran et al., 2009). Notably, the mouse Bcl11a pattern of expression is different, with the XL isoform being already present in FL definitive erythroid cells. This delay in BCL11A-XL in humans could explain the different timing of γ-globin switching in human vs. mouse (Sankaran et al., 2009). The specific γ-globin repression in adult cells is mediated by its binding to the consensus sequence GGTCA, present in several discrete sites within the β-locus (Liu et al., 2018). Among them, the site in the distal CCAAT box region of the β-promoter, which contains the −117 residue, whose G > A mutation causes HPFH, indeed abolishes BCL11A-XL binding (Martyn et al., 2018).

BCL11A is activated by KLF1/EKLF (Borg et al., 2010; Zhou et al., 2010) (Kruppel-Like Factor-1), an erythroid-specific zinc finger TF originally identified because of its ability to bind to CACCC motifs (Miller and Bieker, 1993) and now recognized as a critical regulator of many aspects of erythropoiesis (Perkins et al., 2016). The KLF1 gene knockout in the mouse results in embryonic lethality at around stages E14–E15 due to lethal anemia because of the inability to activate β-globin (Nuez et al., 1995; Perkins et al., 1995) and mutations in the CACCC box of the β-globin promoter that abolish its binding causing thalassemia (Feng et al., 1994). Thus, KLF1 promotes the switching from γ- to β-globin gene expression both directly, by activating the β-globin promoter and indirectly, by activating BCL11A. Finally (Basak et al., 2020), recently demonstrated that the oncofetal protein LIN28B (Piskounova et al., 2011; Shyh-Chang et al., 2013), already known to increase γ-globin expression (Lee et al., 2013, 2015; de Vasconcellos et al., 2014), blocks BCL11A translation. The failure of the above regulatory circuits converging on BCL11A results in elevated HbF.

SOX6 is a HMG box transcription factor, characterized by the presence of a high-mobility group domain (HMG) (Wegner, 1999). SOX6 is expressed in several tissues, including cartilage, testis, neural cells, and erythroblasts (Hagiwara, 2011). Mice with a chromosomal inversion (p100H) disrupting the Sox6 gene, or carrying a targeted inactivation of Sox6 die perinatally, secondary to cardiac or skeletal myopathy (Hagiwara et al., 2000). Sox6-null mouse fetuses and pups are anemic and have defective RBCs (Dumitriu et al., 2006). In erythroid cells, SOX6 has indeed a dual role: it stimulates erythroid cell survival, proliferation, and terminal maturation during definitive murine erythropoiesis (Cantu et al., 2011), and it directly silences embryonic/fetal globin genes (Yi et al., 2006; Xu et al., 2010). The first aspect is mediated by the activation of SOCS3, whose overexpression recapitulates the proliferation arrest imposed by SOX6 (Cantu et al., 2011); the second requires the direct binding and repression of the embryonic ε-globin promoter and the cooperation with BCL11A, via direct physical interaction, to silence γ-globin in adult erythroid cells (Yi et al., 2006; Xu et al., 2010).

The DRED complex (direct repeat erythroid-definitive) is a 540-kDa complex containing the nuclear orphan receptors...
The major transcription factors directly regulating the differential expression of γ-globin during development. (A) The expression of γ-globin in embryonic, fetal, and adult cells is regulated by a large common set of ubiquitous (such as, for example, NF-Y) and erythroid-specific transcription factors, the most important of which are GATA1 and its complexes (Love et al., 2014), NFE2, and TAL1. A very small number of TFs, discussed in the text, are instead directly involved in the time-specific expression of γ-globin and in its deregulation when it persists in the adult. c-MYB activates KLF1 that in turn activates BCL11A, which cooperates with SOX6 in repressing γ-globin. ZBTB7A and TR2/TR4 repress γ-globin independently from BCL11A. Of interest, whereas different γ-globin-specific repressors have been identified so far, little is known about early specific activation of embryo/fetal globin genes, and COUP-TFI is the only γ-globin-specific activator identified so far. The oncofetal LIN28B protein, expressed at high levels in JMML cells concomitantly expressing high γ-globin, blocks BCL11A translation. (B) Schematic representation of the γ-promoter showing the position of HPFH mutations. Created with BioRender.com.

TR2 and TR4, expressed in many tissues, including erythroid cells (Tanabe et al., 2002; Lin et al., 2017). TR2 and TR4 form homodimers or heterodimers binding to GGTCA repeat sequences with variable spacing, a consensus common to non-steroid nuclear receptors (Lee et al., 1998). The double conditional knockout of TR2 and TR4 in mouse erythroid cells results in increased embryonic εy and βh1 globins (Cui et al., 2015). In line with this result, the −117HPFH point mutation, associated with high HbF, reduces TR2/TR4 binding (Tanabe et al., 2002). Finally, TR2 and TR4 have been proposed directly repress GATA1 transcription, suggesting a wider role in erythroid maturation (Tanabe et al., 2007b).

ZBTB7A/LRF is a C2H2 zinc finger TF belonging to the POK (BTB/POZ and Krüppel) group of transcriptional regulators (Davies et al., 1999). ZBTB7A is expressed in various hematopoietic lineages (Maeda, 2016). However, its knockout shows a specific erythroid defect, with mouse embryos dying around E16.5 because of severe anemia, demonstrating that ZBTB7A is required for definitive erythropoiesis (Maeda et al., 2009). Adult-stage knockout of Zbtb7a results in erythropoietin-unresponsive macrocytic anemia, reversed by BIM knockout (Maeda et al., 2009). ZBTB7A specifically represses embryonic and fetal globin gene expression, independently from BCL11A, probably through the interaction with components of the nucleosome remodeling deacetylase (NuRD) complex (Masuda et al., 2016).

**COUP-TFI, THE SELECTIVE ACTIVATOR OF γ-GLOBIN IN YOLK SAC-DERIVED CELLS**

The COUP-TFI gene (Chicken Ovalbumin Upstream Promoter Transcriptional Factor II, also known as NR2F2/ARP1) encodes for an orphan nuclear receptor. Its expression is high in the mesenchymal component of developing organs...
and overall decreases after the completion of organogenesis (Pereira et al., 1995; Lin et al., 2011). Its knockout results in early embryonic lethality (E9.5–E10) (Pereira et al., 1999) caused by defects in angiogenesis and heart development. In the erythroid lineage, Coup-TFII is expressed in the early embryo in the YS and in FL, and it declines around day E12.5 (Filipe et al., 1999; Cui et al., 2015; Fugazza et al., 2020). Although originally identified as ε- and γ-globin gene repressor on the basis of in vitro binding data, its functional role remained elusive (Ronchi et al., 1995; Liberati et al., 2001). Our group recently demonstrated that COUP-TFII is co-expressed with embryonic globin genes in cells of YS origin, where it acts as specific γ-globin activator by binding to the GGTCA motifs present within the β-locus (Fugazza et al., 2020). This last observation opens many questions since the same consensus is bound by BCL11A (Liu et al., 2018) and possibly by TR2/TR4 (Tanabe et al., 2007a) in adult cells. Importantly, COUP-TFII is able to activate γ-globin when overexpressed in adult cells, suggesting once again a very similar cellular environment of fetal vs. adult cells (Fugazza et al., 2020).

### INAPPROPRIATE TIMING OF γ-GLOBIN EXPRESSION IN “ADULT-TYPE” CELLS: HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

HPFH is a benign condition in which γ-globin remains expressed at high levels in adult life (Forget, 1998). HbF can be high in all red blood cells (pancellular HPFH) or restricted to a small subset of erythroid cells (heterocellular HPFH) (Thein et al., 2009). HPFFHs, based on the different types of causative mutations, can be broadly divided in three main categories: deletional HPFH, non-deletional HPFH, and HPFH non-linked with the β-locus. Deletional HPFH is associated with the deletion of large regions of DNA between the γ- and β-globin genes within the β-globin locus, as it happens, for example, in the Sicilian ≈13 kb and in the Italian ≈40-kb deletions1 (Kountouris et al., 2014). Many deletions include the loss of δ- and β-globin genes resulting in (δβ)0-thalassemia and HPFH (Ottolenghi et al., 1982). The molecular mechanism underlying the elevated HbF is complex and involves the concomitant deletion of the β promoter, which removes its competition with the γ promoters for the upstream LCR and for limiting TFs (Forget, 1998). Non-deletional HPFH is caused by point mutations described in both γ-globin promoters. These mutations fall into three distinct clusters: the −200 region, the −175 site, and the distal CCAAT box region, around −115 (Forget, 1998; Martyn et al., 2018), where they either disrupt binding sites for γ-globin repressors or create de novo binding sites for γ-globin activators. For example, the −196 mutation abolishes the binding of ZBTB7A (Martyn et al., 2018), whereas mutations in the distal CCAAT box region impair BCL11A binding (Liu et al., 2018; Martyn et al., 2018). Instead, the −175T > C mutation creates a de novo binding site for the activator TAL1 (Wienert et al., 2015). Despite these evidences, the function of these sequences is more complex.

The −198T > C mutation, for example, although being located within the cluster of HPFH mutations that impair the binding of ZBTB7A, is a gain-of-function mutation creating a binding site for the erythroid activator KLF1 (Wienert et al., 2017). The same is true for the mutation at position −113 A > G, which, although lying within the −115 region bound by the repressor BCL11A, creates a new binding site for the activator GATA1, without altering the BCL11A binding (Martyn et al., 2019).

### THE ROLE OF MODIFIERS LOCI

Genome-wide association studies (GWAS) have revealed other two loci, not linked to the β-locus, consistently associated with HbF levels, and with β-globin disorder severity, across various ethnic backgrounds: a region on 2p (Menzel et al., 2007; Lettre et al., 2008; Uda et al., 2008) and the HBS1L-MYB intergenic region on 6q (Craig et al., 1996; Thein et al., 2007). The 2p region turned out to correspond to BCL11A, and the fine mapping of the single nucleotide polymorphisms associated to HbF within this region led to the identification of the intronic enhancer driving the expression of BCL11A in erythroid cells (Bauer et al., 2013; Canver et al., 2015).

The variants within the HBS1L-MYB intergenic region on 6q impair the binding of LDB1, GATA1, TAL1, and KLF1 to the enhancer controlling c-MYB expression (Stadhouders et al., 2014). c-MYB is the cellular homolog of v-Myb, the avian retroviral oncogene causing myelomas and lymphomas in birds (Wolff, 1996). Of the two major isoforms, isoform 2 (72 kDa) is the dominant one in human erythroid cells (Baker et al., 2010; Wang et al., 2018). In hematopoiesis, c-MYB is expressed in immature cells of all hematopoietic lineages (Wang et al., 2018); in erythropoiesis, it is required for the expansion of erythroid progenitors and must be downregulated to allow differentiation (Emambokus et al., 2003). c-Myb-null murine embryos are normal until E13.5, but by E15, they become severely anemic and die, suggesting that c-Myb is required for definitive erythropoiesis (Mucenski et al., 1991). The reduced c-MYB level has a twofold impact on globin genes. Low c-MYB levels, by accelerating the kinetics of erythroid differentiation, would favor the release of early erythroid progenitor cells still synthesizing HbF (Stamatoyannopoulos, 2005; Jiang et al., 2006). In addition, the reduced activation of KLF1 (Bianchi et al., 2010; Suzuki et al., 2013) by MYB would promote γ-globin expression by reducing BCL11A levels.

### INAPPROPRIATE TIMING OF γ-GLOBIN EXPRESSION IN “FETAL-TYPE” CELLS PERSISTING AFTER BIRTH: THE CASE OF JUVENILE MYELOMONOCYTIC LEUKEMIA

High HbF levels are a hallmark of different leukemias (Sheridan et al., 1976). However, the expression of γ-globin in juvenile myelomonocytic leukemia (JMML) is peculiar. JMML is a rare and aggressive blood cancer of early childhood.

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1https://www.ithanet.eu/
(Loh, 2011; Niemeyer and Flotho, 2019). About 90% of the patients present hyperactivation of the RAS pathway, as a result of mutations in KRAS, NRAS, PTPN11, NF1, or CBL genes, and about 25% of the patients carry chromosome 7 monosomy (Flotho et al., 1999; de Vries et al., 2010). JMML is considered a stem cell disease (Inoue et al., 1987; Busque et al., 1995; Flotho et al., 1999; Cooper et al., 2000). Of interest, increased HbF levels and the presence of fetal red cell traits (Weinberg et al., 1990; de Vries et al., 2010; Helsmoortel et al., 2016a) are present in more than half of JMML patients. These evidences suggested a fetal origin for JMML, confirmed by the retrospective analysis of JMML patient samples collected at birth (Krutz et al., 2005; Matsuda et al., 2010; Stieglicz et al., 2015). However, yolk sac EMPs expressing gain of function PTPN11 mutations recapitulate part of the characteristics of JMML, but they are not able to cause disease in mice (Tarnawsky et al., 2017). Recently, gene expression profiling of JMML samples identified a subgroup characterized by high LIN28B expression and higher HbF levels (Helsmoortel et al., 2016b). LIN28B is an oncofetal protein (Shyh-Chang and Daley, 2013) that induces γ-globin expression (Lee et al., 2013, 2015; de Vasconcellos et al., 2014) and is highly expressed in fetal HSCs (Copley et al., 2013). Interestingly, LIN28B was shown to repress BCL11A-XL by blocking its translation (Basak et al., 2020). Thus, high levels of LIN28B decrease the amount of BCL11A protein, and this results in high HbF. This observation suggests a mechanistic link between LIN28B and high HbF observed in JMML. Whether the 50% of JMML with high HbF arose from a fetal EMP population expressing high LIN28B deserves further investigation.

CONCLUSION

The existence of γ-globin genes is specific to humans and old-world monkeys. Physiologically, γ-globin expression is confined to fetal life, tightly regulated by a complex network of transcription factors (activators and repressors) and co-regulators. Nevertheless, normal subjects present rare F cells, whose nature is still unclear (Rochette et al., 1994). During development, γ-globin genes are expressed in two different types of erythroblasts, with independent origin, the first originating from the transient, yolk sac-derived EMP population and the second from definitive HSCs (McGrath et al., 2011). These two cell types are very similar in their maturational pathway, and adult cells indeed represent a permissive environment for γ-globin expression, as shown by HPFH. Here, alterations in the few specific transcription factors that regulate γ-globin transcription (or in the sequences bound by them within the HBB locus) allow substantial γ-globin expression. Moreover, recent RNA-seq studies on A and F cells from the same healthy donors show that these cells do not significantly differ in the expression of known γ-globin regulators, suggesting that differences in the transcription of globin genes within the HBB locus itself account for the HbF trait (Khandros et al., 2020).

However, new evidence from the study of JMML unveil an alternative possible scenario, where γ-globin genes could be the marker of the fetal origin of these leukemic cells. The discovery that fetal progenitor-derived cells can persist to adulthood and contribute to disease raises the possibility that, especially in childhood malignancies, such as JMML, HbF can indeed be the signature of the fetal origin of cancer cells.

AUTHOR’S NOTE

While this minireview was in press, an article was published by Liu and colleagues (doi: 10.1038/s41588-021-00798-y) showing that BCL11A competes with NF-Y binding to initiate γ-globin repression at the CCAAT box region.

AUTHOR CONTRIBUTIONS

AR conceived and wrote the manuscript. GB and AL contributed with ideas and discussion. SS and AA contributed in the organization of the manuscript. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past co-authorship with one of the authors AR.

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