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Update on fetal hemoglobin gene regulation in hemoglobinopathies

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Abstract

Purpose of the review—The developmental switch from fetal to adult hemoglobin has long fascinated biologists and attracted hematologists given its importance for patients with hemoglobin disorders. New discoveries have reinvigorated the field of globin gene regulation. These results hold promise for improved treatment of the major hemoglobinopathies.

Recent findings—Both genome-wide association studies and traditional linkage studies have identified several genetic loci involved in silencing fetal hemoglobin. BCL11A is a potent silencer of fetal hemoglobin in both mouse and man. It controls the beta-globin gene cluster in concert with other factors. KLF1, a vital erythroid transcription factor, activates BCL11A and assists in coordinating the switch from fetal to adult hemoglobin. A regulatory network of cell-intrinsic and cell-extrinsic factors maintains the epigenetic homeostasis of the beta-globin cluster and accounts for the precise lineage-specific and developmental stage-specific regulation of the globin genes.

Summary—With an improved understanding of pathways involved in the switch from fetal to adult hemoglobin, new targets have emerged for the treatment of the common hemoglobin disorders, sickle cell anemia and beta-thalassemia.

Keywords

fetal hemoglobin; gamma-globin; BCL11A; KLF1; epigenetics

Introduction

A maxim of pediatrics is that developmental stage influences disease expression. In few cases is this more evident than the hemoglobin disorders. Each hemoglobin tetramer is composed of two alpha-like and two beta-like globin polypeptides. Whereas deficient alpha-globin expression causes critical prenatal anemia and fetal hydrops, deficient beta-globin expression is associated with relatively mild or even silent clinical outcomes at birth with disease manifestations delayed until infancy. This disparity is because beta-globin is not a constituent of fetal hemoglobin (HbF), a tetramer of two alpha- and two gamma-globin chains. Around birth, expression of gamma-globin is progressively silenced and beta-globin expression is activated. Individuals with beta-globin disorders have clinical courses whose severity is inversely proportional to the degree of preservation of HbF expression. Rare individuals with beta-hemoglobinopathies and hereditary persistence of fetal hemoglobin (HPFH) do not manifest disease [1]. (Even though fetal hemoglobin has a slightly higher affinity for oxygen than does adult hemoglobin, individuals with HPFH are completely healthy.) The structurally normal gamma-globin is able to substitute for the absent beta-

globin in beta-thalassemia and disrupt sickle hemoglobin-associated polymerization in sickle cell disease. The most successful HbF inducer in the pharmacologic armamentarium is hydroxyurea [2-4]. Hydroxyurea reduces the frequency and severity of sickle cell crises and may forestall end-organ damage [5-7]. However, hydroxyurea has inconsistent effectiveness, requires careful monitoring, and has limited utility for the treatment of beta-thalassemia. There remains an unmet need for more efficient, safe, and tolerable means of HbF induction. In this context, recent advances in the understanding of the developmental regulation of globin gene expression (“hemoglobin switching”) hold promise for the development of novel therapeutic strategies for beta-hemoglobinopathies.

Hemoglobin switching

Five beta-like globin genes reside in a single gene cluster (see Figure 1). *HBE* encodes epsilon-globin, expressed during early embryogenesis in primitive erythrocytes derived from the yolk sac; *HBG1* and *HBG2* encode gamma-globin, expressed from mid-gestation until infancy; and *HBD* and *HBB* encode delta- and beta-globin which compose the minor and major forms of adult hemoglobin, predominating after birth. The acquisition of fetal-stage gamma-globin occurred during primate evolution so in most animals (including the lab mouse) there are only embryonic and adult globin genes. Two hemoglobin switches occur through human ontogeny, initially from embryonic to fetal globins and later from fetal to adult. The clinically relevant aspect of the switch with regard to beta-hemoglobinopathies is the repression of gamma-globin in adult-stage erythroblasts.

Maturation switching describes the finding that initial stages of erythroid differentiation are relatively permissive for gamma-globin expression whereas terminal stages restrict expression to beta-globin [9]. HbF production is enhanced following acute erythropoietic stress, such as after hemorrhage, hemolysis, or recovery from chemotherapy. This HbF surge may be due to the accelerated expansion of early erythroid progenitors that retain gamma-globin expression potential. Cytotoxic agents [10] such as hydroxyurea were first explored for use in HbF induction because of the hypothesis that they might alter the kinetics of erythropoiesis in a manner similar to “stress erythropoiesis.”

HPFH is caused by two classes of mutations, one involving large deletions at the beta-globin cluster and the other involving point mutations in the promoters of the gamma-globin genes. Deletional HPFH is associated with both the loss of repressive intergenic sequences and the juxtaposition of distant enhancers [1,11,12]. Nondeletional forms of HPFH suggest that the promoters of the gamma-globin genes interact with repressive factors.

Conceptual formulations of hemoglobin switching have distinguished between autonomous and competitive modes of control. In autonomous models, gamma-globin is repressed in the adult stage due solely to the trans-acting environment whereas in competitive models, gamma-globin repression depends on the presence of an adjacent beta-globin gene within the cluster. Several lines of evidence support competition of endogenous human globin genes: residual HbF in the adult-stage is not evenly distributed among erythrocytes but rather has a limited expression in a few red blood cells called F-cells; patients with beta-thalassemia typically demonstrate reciprocal elevations of HbF; and heterozygotes with HPFH have decreased expression of beta-globin from the affected but not the unaffected allele [13]. Each beta-globin cluster contains a distal regulatory element (known as the locus control region, or LCR) required for appropriate globin gene expression [14,15]. The LCR participates in long-range looping interactions with individual globin genes, with only one productive LCR-globin gene interaction possible at a given time [16]. However, transgenic mice with an LCR linked solely to gamma-globin in the absence of adjacent globin genes demonstrate partial silencing during development, demonstrating that at least some of the

switch is autonomous [17,18]. Even in competitive models (in which cis-acting interactions are emphasized), some stage-specific trans-acting mechanisms must be invoked to initiate and maintain the switch.

A silencer of gamma-globin

Although intense efforts have focused on the biochemical characterization of globin gene regulation [19-25], major breakthroughs in the field of hemoglobin switching continue to come from human genetics. Perhaps genotype-phenotype correlations are particularly instructive because the problem is limited to control of a single gene within a single lineage.

Genome-wide association studies (GWAS) take advantage of common polymorphisms to relate phenotypes to variation at discrete loci. GWAS, applied to the problem of HbF variation, have yielded consistent and striking findings, across ethnicities and among healthy individuals and those with beta-hemoglobinopathies: a meaningful fraction of the variation in HbF levels is accounted for by common polymorphisms at just three loci [26-32]. These loci include the beta-globin cluster itself (not surprising since rare mutations here result in HPFH), an intergenic interval between the *HBSIL* and *MYB* genes (previously identified by linkage analysis of a family with elevated HbF [33]), and *BCL11A*. *BCL11A*, not previously implicated in hemoglobin switching, was an intriguing candidate, as it is a zinc-finger transcriptional repressor active in other hematopoietic lineages (B lymphoid cells) and expressed in erythroid cells.

Studies from our lab and others have shown that *BCL11A* silences gamma-globin. Knockdown of *BCL11A* enhances HbF production in human erythroid progenitors [34]. Knockout of *BCL11A* in the mouse profoundly delays the single switch from embryonic-to-adult globins as well as silencing of gamma-globin carried on a human beta-globin cluster transgene [35]. *BCL11A* does not occupy the gamma-globin promoter but binds to the LCR as well as intergenic regions in the cluster [34,36,37] previously associated with gamma-globin repression [1,11].

BCL11A associates with various partners within erythroid multiprotein complexes [34,36,37], including the repressive nucleosome remodeling and deacetylase complex (NuRD), GATA1, the erythroid master regulator, and SOX6, a transcription factor previously shown to repress embryonic-globins in mice [38-41]. Unlike *BCL11A*, SOX6 directly occupies the gamma-globin promoter and double knockdown of *BCL11A* and SOX6 has an additive effect on HbF derepression, suggesting that *BCL11A* may exert part of its gamma-globin repression with SOX6 [36].

Trans-acting factors important in globin gene regulation must themselves be developmentally regulated to be effectors of the physiologic switch. In the adult stage, *BCL11A* is expressed at high levels whereas in embryonic and fetal stages, its expression level is much lower and shorter isoforms predominate [34,35]. Future studies will help determine the extent to which erythroid *BCL11A* is subject to post-translational control such as proteolytic cleavage or SUMOylation [34,42,43]. *BCL11A* is also under transcriptional control [36], with its locus occupied by GATA1 [37]. Therefore *BCL11A* appears to be a new therapeutic target for HbF induction. One issue will be the degree to which inhibition of *BCL11A* has non-erythroid effects, such as in B cells.

A master erythroid regulator

The next clue in the hemoglobin switching puzzle again came from human genetics. The first hint was a patient with congenital dyserythropoietic anemia (CDA) found to have persistently elevated HbF and a mutation in the *KLF1* gene causing a single amino acid

substitution in the second zinc finger of this transcription factor [44]. Recently, a mouse mutation called *Nan* (for neonatal anemia) was mapped to a substitution of the equivalent zinc finger residue within *KLF1* [39,45]. Heterozygous *Nan* mice have chronic hemolytic anemia and persistently elevated embryonic globin expression [39]. The *Nan* allele is associated with more severe phenotypes than the simple *KLF1* knockout indicating neomorphic properties. Of note, individuals with loss-of-function *KLF1* mutations were known to display the In(Lu) phenotype (disturbed expression of certain blood group antigens) but were not reported to have deranged globin gene expression [46]. Subsequently, a novel form of HPFH in a Maltese kindred was mapped to heterozygous nonsense mutations in the first zinc finger of *KLF1* [47]. Unlike the neomorphic *Nan* mutation, Maltese HPFH appears to be due to a haploinsufficient *KLF1* allele. Further characterization will be required to compare this allele to those associated with the In(Lu) phenotype.

Knockdown of *KLF1* in human erythroid progenitors derepresses HbF [47,48]. *KLF1* (also known as EKLF) was first characterized as an erythroid transcription factor binding to CACCC motifs [49]. Thalassemia intermedia patients with mutations in the CACCC box of the beta-globin promoter were described nearly thirty years ago [50], and these patients demonstrate significant elevation of HbF [51]. *KLF1* is a potent activator of beta-globin in vivo, as demonstrated by knockout mice that succumb to fatal beta-thalassemia [52-54]. Careful observation of these mice (and those bearing a human beta-globin cluster transgene) prior to their death demonstrates delayed silencing of mouse embryonic globin and human transgenic gamma-globin genes in the homozygous and heterozygous *KLF1* mutants [55,56].

Surprisingly, gene expression profiling of erythroid progenitors in patients with Maltese HPFH demonstrated decreased expression of *BCL11A* [47]. Knockdown of *KLF1* led to decreased *BCL11A* in normal erythroid progenitors and reexpression of *KLF1* rescued *BCL11A* expression in erythroid progenitors of patients with Maltese HPFH [47]. In a parallel study, mice with a hypomorphic *KLF1* allele demonstrated delayed silencing of murine embryonic globins (and transgenic human gamma-globin) and reduced *BCL11A* [48]. *KLF1* occupies the *BCL11A* promoter [47,48].

These studies suggest a model in which *KLF1* has dual functions. First, it influences gamma-globin silencing by providing the beta-globin gene a competitive advantage, and second, it activates the gamma-globin silencer *BCL11A*. This seeming reconciliation of competitive and autonomous models begs the question of how *KLF1* itself is developmentally regulated. Although *KLF1* modestly increases in concentration between embryonic and adult stages [57,58], it is active and competent for high-level beta-globin expression in the embryonic stage [54,59] suggesting that layers of its regulation remain obscure. Furthermore in mice carrying a human beta-globin cluster transgene, the magnitude of gamma-globin derepression observed in *KLF1* deficiency is much less than that seen in *BCL11A* deficiency implying additional relevant inputs to *BCL11A* regulation. *KLF1* not only coordinates globin gene expression but also is a regulator involved in supporting many aspects of erythroid biology [60-62]. Various KLF factors play redundant and opposing roles in vivo [59,63]. Therefore interventions aimed at inducing HbF by inhibiting *KLF1* might have a narrow therapeutic window.

The other locus highlighted by GWAS is the intergenic interval between *HBS1L* and *MYB*. While studies have conflicted as to which of these two genes regulates HbF [64-66], the weight of evidence points to c-Myb, an erythroid transcription factor [67]. A recent study demonstrated that knockdown of c-Myb during erythroid differentiation of human hematopoietic progenitors was associated with decreased expression of *KLF1* [68]. The

simple model implied by this study that c-Myb represses gamma-globin via KLF1 activation of BCL11A awaits experimental support.

The importance of epigenetics

Chromatin modifying activities participate in the hemoglobin switch. The gamma-globin gene becomes methylated in the adult stage while the beta-globin gene remains unmethylated [69,70]. The gamma-globin promoter is occupied by multiprotein repressive complexes including the arginine methyltransferase PRMT5, the DNA methylating enzyme DNMT3A, the lysine methyltransferase SUV4-20h1, the serine/threonine kinase CK2alpha, and components of NuRD [71,72]. Teasing out the role of the sundry chromatin modifying activities residing at the beta-globin cluster remains a challenge. Recent approaches have begun to scrutinize these complexes with surprising results. NuRD appeared to be an excellent candidate for mediating repression of gamma-globin, in concert with BCL11A [34] and GATA1-FOG1 [25], among other partners. A FOG1 mutation engineered to lack the NuRD binding domain and knocked-in to the endogenous locus does not impair transgenic human gamma-globin (or endogenous murine embryonic globin) silencing but paradoxically leads to decreased beta-globin expression [73]. This suggests a perplexing model in which the GATA1-FOG1-NuRD complex is required for globin activation (rather than repression). Whether NuRD plays this unforeseen role within other complexes remains to be seen.

Another insight has come from study of PRMT1, an arginine methyltransferase linked to activation of beta-globin [74]. Surprisingly, knockdown of FOP (Friend of PRMT1), a recently identified PRMT1 partner, causes significant derepression of gamma-globin [75]. FOP expression rises from fetal to adult stages, suggesting it might participate in the physiologic switch. Numerous questions remain regarding the function of this factor, including whether these effects are through PRMT1, other methyltransferases, or via an altogether different pathway.

The physiologic switch depends on the interplay between the erythroblast-intrinsic machinery and signals emanating from the stage-specific niche. A population of fetal hepatic stromal cells has recently been identified that support hematopoietic cells by expressing cytokines such as SCF, which are differentially regulated in fetal liver compared to bone marrow [76]. SCF promotes maintenance of HbF expression [77,78] and this cytokine alters the distribution of histone marks within the beta-globin cluster [79]. The SCF receptor decreases on erythroid progenitors from fetal to adult life, targeted by the adult stage-specific microRNAs miR-221 and miR-222 [80].

Beginnings of a network

A host of new players implicated in repressing HbF present as therapeutic targets for the treatment of beta-hemoglobinopathies (see Figure 2). A critical task will be to place these factors into a unified hierarchy, comparing various regulators in terms of quantitative effects on globin expression, relationships to other factors, non-globin effects on erythropoiesis, and impacts outside of the erythroid lineage. A granular network of HbF regulatory pathways will help prioritize the most promising leads for further clinical development.

Key regulators of the globin switch may serve as direct targets for small molecules. DNA-binding transcription factors such as BCL11A or KLF1 have been considered challenging targets for drug development although recent advances may extend the reach of small molecules [81]. Since these transcription factors cooperate with chromatin modifying activities, traditional enzyme inhibitors may also have a role in HbF induction. The imperative will be to define activities that have limited effects outside of hemoglobin

switching. Demethylating agents have been explored; promising results with 5-azacytidine in a beta-thalassemia patient were noted nearly 30 years ago [82]. Decitabine, a demethylator with a more encouraging safety profile, has shown impressive HbF induction in primate models [43] and remains in clinical development. Histone deacetylase inhibitors also have potential to induce HbF [83-85]. A recent study integrated high-throughput cell-based screening, an extensive library of HDAC inhibitors, and RNA interference, to define HDAC1 and HDAC2 as the specific deacetylases responsible for HbF repression [86]. Selective HDAC modulators might achieve HbF induction while avoiding toxicity. Advanced chemical biology methods may allow for agnostic screens to probe undiscovered pathways influencing the globin switch.

Genetic and cellular therapeutics may also have a role. Such strategies could include the in vivo application of RNA interference [87], engineered transcription factors [88], or the use of stem cells [89]. Recently an artificial transcription factor targeting the gamma-globin promoter was shown to derepress HbF [90]. This form of gene correction in trans is one of many potential strategies to reverse the switch.

Conclusion

The study of hemoglobin switching was reinvigorated by the recent discovery of BCL11A as a potent silencer of gamma-globin. The finding that KLF1 promotes fetal hemoglobin repression through activation of both beta-globin and BCL11A assimilates competitive and autonomous models. A tentative network of fetal hemoglobin regulation materializes, involving c-Myb, KLF1, and BCL11A, and integrating epigenetic mechanisms with extrinsic influences. Despite great progress in the field, only half the variance in fetal hemoglobin levels has been explained, suggesting uncharted insights will help clarify the system and prioritize clinical development.

One and two bulleted annotations

*Aerbajinai, W., Zhu, J., Kumkhaek, C., Chin, K. and Rodgers, G. P. SCF induces gamma-globin gene expression by regulating downstream transcription factor COUP-TFII 2009

The authors demonstrate that SCF suppresses COUP-TFII, a known repressor of gamma-globin, via the ERK pathway. This study uniquely addresses the study of cell-extrinsic and cell-intrinsic mechanisms of gamma-globin regulation.

**Bianchi, E., Zini, R., Salati, S., et al. c-Myb supports erythropoiesis through the transactivation of KLF1 and LMO2 expression 2010

Using a knockdown approach in human hematopoietic progenitors, the authors show that c-Myb promotes erythropoiesis through activation of KLF1 and LMO2 (a component of the GATA1-TAL1 complex). This result is intriguing given the association of polymorphisms at the *HBS1L-MYB* intergenic interval with HbF level. The model implied by this study that c-Myb represses gamma-globin via KLF1 activation of BCL11A awaits experimental support.

**Borg, J., Papadopoulos, P., Georgitsi, M., et al. Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin 2010

This important study maps a novel form of HPFH to mutations in *KLF1*. Surprisingly, BCL11A is deficient in these patients due to insufficient activation by KLF1. KLF1 appears to effect hemoglobin switching through both competitive and autonomous modes. It remains unclear how various *KLF1* mutations result in such disparate erythroid phenotypes.

*Bottardi, S., Ross, J., Bourgoin, V., et al. Ikaros and GATA-1 combinatorial effect is required for silencing of human gamma-globin genes 2009

The authors demonstrate that Ikaros, previously reported as a repressor of gamma-globin, is required for GATA1 occupancy of the gamma-globin promoter and the appropriate distribution of chromatin activities and repressive marks.

**Bradner, J. E., Mak, R., Tanguturi, S. K., et al. Chemical genetic strategy identifies histone deacetylase 1 (HDAC1) and HDAC2 as therapeutic targets in sickle cell disease 2010

This report uses chemical screening and RNA interference to demonstrate the role of HDAC1 and HDAC2 in gamma-globin repression. The results suggest that selective HDAC inhibitors might derepress HbF while limiting systemic toxicity. Moreover, the study highlights the utility of high-throughput chemical genetics.

*Chin, J., Singh, M., Banzon, V., et al. Transcriptional activation of the gamma-globin gene in baboons treated with decitabine and in cultured erythroid progenitor cells involves different mechanisms 2009

The authors extend their previous studies on decitabine in baboons, showing that drug treatment causes demethylation of the gamma-globin promoter as well as the acquisition of active histone marks. Since it possesses endogenous gamma-globin (unlike the mouse), the baboon is a key animal model for exploring new therapeutics.

**Chou, S., Lodish, H. F. Fetal liver hepatic progenitors are supportive stromal cells for hematopoietic stem cells 2010

The authors isolated rare fetal liver cells that could support the expansion of fetal hematopoietic stem cells. These stromal cells were shown to be alpha-fetoprotein-expressing hepatic stem or progenitor cells. The stromal cells expressed cytokines differentially regulated between fetal liver and adult bone marrow. This study highlights fundamental distinctions between fetal and adult hematopoiesis.

**Gabbianelli, M., Testa, U., Morsilli, O., et al. Mechanism of human Hb switching: a possible role of the kit receptor/miR-221-222 complex 2010

The authors show that kit (the SCF receptor) is progressively silenced during development, suggesting that SCF may participate in the physiological hemoglobin switch. Moreover, they demonstrate that the erythroid microRNAs miR-221 and miR-222 target kit and are more highly expressed in the adult compared to fetal stage. MicroRNAs surface as a therapeutic target for HbF induction.

*Gazouli, M., Katsantoni, E., Kosteas, T. and Anagnou, N. P. Persistent fetal gamma-globin expression in adult transgenic mice following deletion of two silencer elements located 3' to the human Agamma-globin gene 2009

Using detailed transgenic studies, the authors describe a cis element repressing gamma-globin expression in the adult stage. Future studies using similar transgenes will help define the interactions that establish hemoglobin switching in vivo; for example, it is possible that BCL11A exerts its gamma-globin repressive effect via interactions with such cis elements.

*Heruth, D. P., Hawkins, T., Logsdon, D. P., et al. Mutation in erythroid specific transcription factor KLF1 causes Hereditary Spherocytosis in the Nan hemolytic anemia mouse model 2010

This study, like that of Siatecka et al, maps the *Nan* mutation to *KLF1*. The authors propose a model in which this mutant encodes an abnormal KLF1 protein with altered DNA-binding properties.

**Jawaid, K., Wahlberg, K., Thein, S. L. and Best, S. Binding patterns of BCL11A in the globin and GATA1 loci and characterization of the BCL11A fetal hemoglobin locus 2010

This important report using high-resolution ChIP-chip in human adult erythroid progenitors confirms that BCL11A, although occupying the beta-globin cluster, occupies neither the gamma-globin nor the beta-globin promoter. Rather BCL11A occupies important regulatory regions including the LCR and several loci in the gamma-delta intergenic interval. Furthermore, BCL11A and GATA-1 often appear to share co-occupancy, indicating they may participate in a multi-subunit complex. GATA1 binds to intronic regions of the BCL11A gene, adjacent to HbF-associated SNPs, suggesting that these polymorphisms mark key regulatory elements.

*Lebensburger, J. D., Pestina, T. I., Ware, R. E., Boyd, K. L. and Persons, D.A. Hydroxyurea therapy requires HbF induction for clinical benefit in a sickle cell mouse model 2010

This report supports the concept that hydroxyurea ameliorates sickle cell disease via HbF induction. The authors demonstrate no salutary effects of hydroxyurea on anemia or end-organ compromise in a murine model of sickle cell disease (which lacks gamma-globin) whereas ectopic gamma-globin expression was able to reverse the disease phenotype.

*Miccio, A., Blobel, G. A. Role of the GATA-1/FOG-1/NuRD pathway in the expression of human beta-like globin genes 2010

With knock-in mice bearing a FOG1 mutation that ablates the NuRD binding domain, the authors surprisingly show that the GATA1-FOG1-NuRD complex promotes globin activation (rather than repression). This study shows the challenges in deconvoluting multiprotein complexes.

*Rank, G., Cerruti, L., Simpson, R. J., Moritz, R. L., Jane, S. M. and Zhao, Q. Identification of a PRMT5-dependent repressor complex linked to silencing of human fetal globin gene expression 2010

The authors show that the lysine methyltransferase SUV4-20h1 is a member of the PRMT5 complex at the gamma-globin promoter and that knockdown of SUV4-20h1 results in derepression of gamma-globin. This study reveals that chromatin-modifying activities (i.e. “druggable targets”) actively participate in hemoglobin switching.

**Sankaran, V. G., Xu, J., Ragoczy, T., et al. Developmental and species-divergent globin switching are driven by BCL11A 2009

This report demonstrates that BCL11A participates in physiologic hemoglobin switching. Profound derepression of transgenic human gamma-globin and endogenous embryonic globins accompanies BCL11A deficiency in mice. Significant delay in hemoglobin switching occurs in heterozygous animals showing that BCL11A is a quantitative silencer of gamma-globin. BCL11A expression is developmentally regulated although the mechanisms of this remain incompletely characterized.

**Siatecka, M., Sahr, K. E., Andersen, S. G., Mezei, M., Bieker, J. J. and Peters, L. L. Severe anemia in the Nan mutant mouse caused by sequence-selective disruption of erythroid Kruppel-like factor 2010

The ENU-induced *Nan* (neonatal anemia) allele is mapped to a missense mutation of *KLF1* affecting an equivalent residue in the second zinc finger as a human *KLF1* mutation causing congenital dyserythropoietic anemia and elevated HbF. Unlike the *KLF1*^{+/-} mouse, the *Nan* heterozygote is anemic with persistent embryonic globin expression, and the *Nan* homozygote is embryonic lethal at an earlier stage than is the *KLF1* null mouse, suggesting that the *Nan* allele has neomorphic or dominant negative properties, apparently due to altered DNA-binding specificity. This study confirms the role of KLF1 in hemoglobin switching and highlights that various *KLF1* alleles have disparate erythroid phenotypes.

*Spyrou, P., Phylactides, M., Lederer, C. W., et al. Compounds of the anthracycline family of antibiotics elevate human gamma-globin expression both in erythroid cultures and in a transgenic mouse model 2010

This study demonstrates that anthracyclines currently used clinically for the treatment of malignancies can at low-doses induce HbF. It remains to be determined if the HbF-inducing activity of these agents can be dissociated from their cytotoxicity.

*Sripichai, O., Kiefer, C. M., Bhanu, N. V., et al. Cytokine-mediated increases in fetal hemoglobin are associated with globin gene histone modification and transcription factor reprogramming 2009

The authors extend their previous findings that the cytokines SCF and TGF-beta derepress gamma-globin in ex vivo cultured human erythroblasts and demonstrate that these cytokines cause changes in chromatin occupancy at the beta-globin cluster. This study provides an example of how cell-extrinsic influences may bear on epigenetic regulation.

*van Dijk, T. B., Gillemans, N., Pourfarzad, F., et al. Fetal globin expression is regulated by Friend of Prmt1 2010

In this brief report, the authors show that FOP (Friend of PRMT1) is a repressor of gamma-globin in adult erythroid cells. It is unclear if FOP mediates this effect through its namesake PRMT1, other arginine methyltransferases such as PRMT5, or through a methyltransferase-independent pathway.

*Wilber, A., Tschulena, U., Hargrove, P. W., et al. A zinc-finger transcriptional activator designed to interact with the gamma-globin gene promoters enhances fetal hemoglobin production in primary human adult erythroblasts 2010

The authors designed an artificial transcription factor composed of a transcriptional activation domain and a gamma-globin promoter DNA binding domain. This construct led to significant activation of HbF in human hematopoietic progenitors. This creative application of gene correction in trans demonstrates a novel strategy for genetic therapeutics.

**Xu, J., Sankaran, V. G., Ni, M., et al. Transcriptional silencing of {gamma}-globin by BCL11A involves long-range interactions and cooperation with SOX6 2010

This report, along with that of Jawaid *et al*, uses high-resolution ChIP-chip to show that BCL11A binds to the beta-globin cluster not at the gamma-globin or beta-globin genes themselves but at distant sites, including the LCR and gamma-delta intergenic intervals. BCL11A is shown to promote long-range interactions between the LCR and *HBB* at the

expense of *HBG*. BCL11A also interacts with SOX6, a known repressor of murine embryonic globins. SOX6 is shown to repress gamma-globin in adult human erythroid progenitors and to occupy the gamma-globin promoter. Therefore, BCL11A may act with various partners to effect repression of gamma-globin. Future experiments will help characterize the critical multiprotein complexes and beta-globin regulatory sequences through which BCL11A represses gamma-globin.

**Zhou, D., Liu, K., Sun, C. W., Pawlik, K. M. and Townes, T. M. KLF1 regulates BCL11A expression and gamma- to beta-globin gene switching 2010

This study characterizes mice with hypomorphic alleles of *KLF1* with delayed hemoglobin switching. Similar to the Maltese HPFH kindred, these mice have decreased levels of BCL11A and KLF1 binds to the BCL11A promoter. An important avenue for future study is defining the developmental transition that promotes KLF1-dependent BCL11A activation.

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Figure 1. Developmental regulation of the beta-globin locus

Top, relative expression of beta-like globin genes through ontogeny. Bottom, schematic of the beta-globin locus, including five genes and a distal regulatory element known as the locus control region (LCR), Graph modified from [8].

Figure 2. An emerging network of fetal hemoglobin regulation

In the adult stage, the beta-globin gene, engaged in long-range looping interactions with the LCR, is expressed while the gamma-globin gene is repressed. Regulators of this process comprise therapeutic targets for beta-hemoglobinopathies. Positive and negative interactions are denoted by pointed and blunt arrows, respectively. Dotted lines indicate relationships with a lower level of experimental support.