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Epigenetic and genetic mechanisms in red cell biology

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Abstract

Purpose of review—Erythropoiesis, in which hematopoietic stem cells (HSCs) generate lineage-committed progenitors that mature into erythrocytes, is regulated by numerous chromatin modifying and remodeling proteins. We will focus on how epigenetic and genetic mechanisms mesh to establish the erythroid transcriptome and how studying erythropoiesis can yield genomic principles.

Recent findings—*Trans*-acting factor binding to small DNA motifs (*cis*-elements) underlies regulatory complex assembly at specific chromatin sites, and therefore unique transcriptomes. As *cis*-elements are often very small, thousands or millions of copies of a given element reside in a genome. Chromatin restricts factor access in a context-dependent manner, and *cis*-element-binding factors recruit chromatin regulators that mediate functional outputs. Technologies to map chromatin attributes of loci *in vivo*, to edit genomes and to sequence whole genomes have been transformative in discovering critical *cis*-elements linked to human disease.

Summary—*Cis*-elements mediate chromatin-targeting specificity, and chromatin regulators dictate *cis*-element accessibility/function, illustrating an amalgamation of genetic and epigenetic mechanisms. *Cis*-elements often function ectopically when studied outside of their endogenous loci, and complex strategies to identify nonredundant *cis*-elements require further development. Facile genome-editing technologies provide a new approach to address this problem. Extending genetic analyses beyond exons and promoters will yield a rich pipeline of *cis*-element alterations with importance for red cell biology and disease.

Keywords

chromatin; *cis*-element; epigenetics; erythropoiesis

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

The progressive transition of a nucleated erythroblast into an enucleated erythrocyte requires profound morphological and functional changes to ensure the generation of billions of erythrocytes daily [1,2]. As this process involves massive chromatin structure reconfiguration, there is considerable interest in identifying the regulators, how they are integrated to yield a vital network, and factors/signals that control the regulators. The proteins mediating chromatin transitions are often referred to as 'epigenetic regulators'. Although the semantics of equating 'epigenetics' with chromatin mechanisms is hotly debated, these factors may generate memory that dictates the daughter cell transcriptome and phenotypes. Moreover, the DNA methylation epigenetic mark 5-methylcytosine is inextricably linked to chromatin mechanisms [3]. We shall focus on the role of chromatin regulators in generating the erythroid transcriptome. As *cis*-elements recognized by activators and repressors underlie chromatin-targeting specificity, their genetic integrity is crucial to ensure normal chromatin landscapes. On the basis of the amalgamation of genetic and epigenetic mechanisms, ascribing genetic or epigenetic components to a biological process can be murky.

Large numbers of erythroid cells representing distinct maturation states can be isolated, and powerful models exist for studying erythropoiesis and erythroid cell function [1,2]. As such, erythroid cells are ideal for investigating chromatin control of genome function, development and homeostasis. Erythropoiesis is controlled by a restricted cohort of lineage-specific master transcriptional regulators functioning in concert with broadly expressed factors. The founding member of the GATA-binding protein (GATA) transcription factor family GATA-1 [4,5] and Kruppel-like factor 1 (KLF1) [6] exemplify core determinants of the erythroid transcriptome that regulate both shared and unique target genes. GATA-2 [7,8], runt-related transcription factor 1 (RUNX1) [9,10] and T-cell acute lymphocytic leukemia protein 1 (TAL1) [11] have pivotal roles to promote the genesis and/or function of hematopoietic stem/progenitor cells (HSPCs), although TAL1 is also expressed and functions in erythroid cells [12].

Massive efforts utilizing genome-wide technologies have mapped transcription factor, coregulator and chromatin landscapes in living cells [13,14,15▪▪,16,17], including erythroid cells [18–22,23▪,24,25,26▪]. An ongoing challenge is to leverage these datasets into innovative discoveries to explain epigenetic mechanisms underlying erythroid precursor cell development into erythrocyte, and how these mechanisms are impacted by stress and pathophysiological states.

TRANSCRIPTIONAL CONTROL OF ERYTHROID CELL DEVELOPMENT AND FUNCTION

Although the dual zinc finger transcription factors GATA-1 and GATA-2 share a similar DNA binding domain [27], they differ in significant ways. They are differentially expressed during hematopoiesis, with GATA-2 expressed predominantly in HSPCs and GATA-1 expressed in erythroid, megakaryocytic, mast and eosinophil cells [5,7,8,28]. They have distinct functions to mediate generation, proliferation and/or survival of hematopoietic cell

types [7,8,29,30,31▪,32▪]. GATA-2 induces hematopoietic stem cell (HSC) generation from hemogenic endothelium in the aorta gonad mesonephros region of the embryo and regulates HSPC function [31▪–34▪]. By contrast, hemogenic endothelium and HSCs express little to no GATA-1. GATA-1 functions in erythroid precursors to promote erythroid cell development and maturation [29]. GATA-1 and GATA-2 have distinct biochemical attributes, for example, GATA-2 is less stable than GATA-1 [35], and GATA-1 selectively requires the coregulator friend of GATA-1 (FOG-1) for many of its actions [36,37]. Finally, GATA-1 and GATA-2 differentially regulate target gene transcription [38,39].

Despite their differences, GATA-1 and GATA-2 actions are intricately linked. GATA-1 is upregulated at an early stage of erythropoiesis and directly represses *Gata2* expression [38,40]. In erythroid precursors, GATA-2 occupies five sites at the active *Gata2* locus, indicative of positive autoregulation [40–42]. As GATA-1 levels/activity rise, GATA-1 displaces GATA-2 from genomic sites – a process termed GATA switching [38–40]. GATA-1 upregulation in erythropoiesis occurs during the early transition (‘S0 to S1’) involving commitment to terminal differentiation and dramatic chromatin reconfiguration [43]. Given the GATA-2 function in hemogenic endothelium and HSPCs, and GATA-1 promotion of erythropoiesis, GATA switches control erythroid precursor cell differentiation into erythrocytes. Not all GATA-1/GATA-2 chromatin occupancy sites are GATA switch sites, as certain sites are occupied preferentially or exclusively by GATA-1 or GATA-2 [18,26▪,44].

As GATA-1 upregulation induces GATA switches, it is instructive to consider the factors/signals that control GATA-1 expression and activity. Coregulators mediate GATA-1 activity in a context-dependent manner [20,37,45▪,46–48]. GATA-1-mediated activation and repression is facilitated by FOG-1 at many target loci [37,49]. This multi-zinc finger protein binds the GATA-1 N-terminal zinc finger and appears to lack DNA binding activity [37,50]. The GATA-1 C-terminal zinc finger binds to the GATA motif (A/TGATAA) [51,52], which in a chromatin context has the consensus (C/G)(A/T)-GATAA(G/A/C)(G/A/C) [18]. FOG-1 copurifies with the NuRD chromatin remodeling complex, and NuRD mediates certain GATA-1 functions [53,54]. FOG-1 facilitates GATA-1 chromatin occupancy at select sites [55,56], precludes GATA-1 occupancy at others [23▪] and facilitates GATA switches [55]. GATA-1 occupies several thousand genomic sites, with the highest frequency at introns and sites distal to promoters [18–21]. GATA-1 and GATA-2 form a multimeric transcriptional regulatory complex with TAL1, LIM domain only protein 2 (LMO2) and LIM domain-binding protein 1 (LDB1) [57]. GATA-1-occupied and GATA-2-occupied loci are commonly associated with one or more of these factors [20,58–62,63▪▪], and enrichments in histone H3K4me1 [21]. LDB1 and LMO2 can contribute to GATA-1-mediated activation and repression [47]. The Brahma-related gene 1 (BRG1) component of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex induces chromatin accessibility at GATA-1-occupied enhancers [64,65]. Interferon regulatory factors colocalize with GATA-1 at certain sites and confer stage-specific transcription [66]. E-twenty-six (ETS) transcription factors can cooccupy chromatin with GATA-1/GATA-2, influencing their activities [44,67,68]. Given the numerous ETS factors, it will be instructive to consider how multiple ETS factors in the same cell influence GATA factor function.

Although it is in vogue to study factor colocalization at endogenous sites, extrapolating chromatin immunoprecipitation (ChIP) data to function can be precarious, as formaldehyde cross-linking might reflect nonredundant or redundant function, or an interaction with no functional significance. Knockdown studies in a genetic complementation assay in GATA-1-null G1E cells represent a powerful approach to identify functional determinants of GATA-1-mediated transcriptional regulation. G1E cells, which resemble normal proerythroblasts, were derived from mouse embryonic stem cells via disruption of the *Gata1* locus [69]. Expression of an estrogen receptor ligand binding domain fusion to GATA-1 allows one to rapidly activate GATA-1, which induces a normal erythroid program [40,70] that recapitulates a physiologically relevant window of erythroid maturation [70].

Studies in G1E cells indicate that the mechanistic requirements for GATA-1-mediated activation and repression differ in distinct contexts. GATA-1-regulated loci can be sensitive or insensitive to FOG-1, the NuRD component Mi2β and the histone H3K20 monomethyltransferase SetD8 [45▪]. The differential locus sensitivity to partial factor knockdowns (50–80%) may reflect overt qualitative differences or locus-specific concentration requirements for the factors. The context-dependent FOG-1 requirement for GATA-1 activity was first demonstrated using GATA-1 mutants (V205G or V205M) impaired in FOG-1 binding [37]. As these mutants do not eliminate FOG-1 binding, alternative strategies have been devised, including genetic complementation in FOG-1-null hematopoietic precursor cells [71]. However, these cells lack certain factors required for erythroid gene expression. Multiple lines of evidence using these strategies provide evidence for differential FOG-1 requirements at distinct loci.

Of high relevance to GATA-1 function is its capacity to regulate local chromatin structure [72–74] and to induce long-range chromatin looping [75]. GATA-1 induces a chromatin loop between the β-globin locus control region (LCR) and the downstream *βmajor* promoter [75]. FOG-1 is required for GATA-1-dependent looping, at least at the limited number of loci examined. This might relate to its activity to facilitate GATA-1 occupancy or an unidentified mechanism. LDB1 [76] and BRG1 [77] also promote this chromatin loop. GATA-1 expels the β-globin locus from the nuclear periphery concomitant with looping [78,79], and expulsion characterizes primary erythroid cell maturation [80]. As the nuclear periphery can create an inhospitable environment for transcription [81], expulsion may relocate the locus into a favorable environment to generate high levels of β-globin. Tiling the β-globin locus and neighboring sequences with bacterial artificial chromosome probes indicated that expulsion is restricted to the β-globin locus and does not involve a considerably broader region [79]. KLF1 promotes the LCR–*βmajor* promoter loop [82], contributes to expulsion [79] and has a broader role in conferring subnuclear positioning of genes [83]. Recent evidence indicates that GATA-1 functions as a mitotic ‘bookmark’ to ensure the stable maintenance of lineage-specific gene expression throughout development [84▪▪].

Given the instrumental role of GATA-1 to establish/maintain the erythroid cell transcriptome and to promote erythropoiesis, defining mechanisms that control GATA-1 expression/activity are very important. *Gata1* expression in erythroid cells is regulated by an upstream GATA motif-containing enhancer [85]. Targeted deletion of this *cis*-element, which contains GATA and CACCC motifs, yields an erythroid maturation defect only when the *NeoR* gene

remains at the targeted site; *NeoR* excision is associated with normal erythropoiesis [86]. *In vivo* footprinting and overexpression analysis in zebra-fish suggest that GATA-1 autoregulates its own expression [87,88]. PBX and MEIS1 act upstream of GATA-1 in erythropoiesis [89], and Biklf [90] and ZBP-89 [91] promote *Gata1* expression in zebrafish. In an embryonic stem cell differentiation system, bone morphogenetic protein signaling induces *Gata1* [92]. The sumo ligase PIAS1 represses *Gata1* in HSCs [93]. Despite this entourage of factors, considerable work is required to achieve a coherent model for how their activities are integrated through *Gata1 cis*-elements.

The myeloid transcription factor PU.1 is expressed reciprocally with GATA-1 and represses GATA-1 activity [94]. PU.1 downregulation enhances GATA-1 activity concomitant with, or as a prelude to, *Gata2* repression, and GATA-1 represses PU.1 expression [95]. Phosphorylation, sumoylation and acetylation regulate GATA-1 activity. Although GATA-1 is multisite phosphorylated [96], with Ser302 phosphorylated by Akt [97], how phosphorylation influences GATA-1 activity is unclear. GATA-1 sumoylation at Lys137 facilitates its regulation of FOG-1-dependent target genes and expulsion of the loci from the nuclear periphery [78]. As FOG-1 [55] and GATA-1 multisite acetylation [98] promote GATA-1 chromatin occupancy, mechanisms regulating FOG-1 levels/activity and the acetylation pathway dictate GATA-1 activity, and therefore GATA switches. FOG-1 is multisite sumoylated [99] and presumably regulated by diverse post-translational modifications.

Even though GATA-1 and GATA-2 control distinct processes during hematopoiesis, they confer primitive erythroblast survival redundantly [100]. GATA-1 and GATA-2 can colocalize with a cohort of factors [TAL1, lympoblastomic leukemia 1 (LYL1), LMO2, RUNX1, ETS-related gene (ERG) and friend leukemia integration 1 (FLI1)] that have important functions to control hematopoiesis [62,101–104]. Coregulators including p300 [105], MED1 [106] and HDAC3/4 [105,107] have been reported to mediate GATA-2 function. Although GATA-2 is acetylated [105,108] and phosphorylated [109], and MAP kinase and Akt phosphorylate GATA-2 [109,110], the modification sites and consequences are unclear.

In addition to established GATA-1 and GATA-2 coregulators, other chromatin modifying enzymes are implicated in erythropoiesis and/or erythroblast function. The histone methyltransferases Mll, Dot1L, Ezh2 and SetD8 regulate hematopoiesis and/or hematopoietic cell function [45▪,111–113]. The lysine-specific demethylase LSD1 [114] regulates erythroid progenitor differentiation by repressing genes associated with HSPCs [115,116]. As histone marks and DNA methylation dramatically change upon erythroid maturation [117,118], elucidating molecular determinants of these patterns will yield important mechanistic insights. Attempting to unravel how factors control specific loci without knowing the requisite *cis*-elements is analogous to embarking on the construction of a sophisticated architectural structure devoid of a blueprint.

IDENTIFYING AND ANALYZING NONREDUNDANT *CIS*-ELEMENTS IN COMPLEX GENOMES

ChIP-seq commonly reveals several thousand factor occupancy sites with only a small fraction of the *cis*-elements occupied. Although thousands of GATA motifs exist in a genome, each of which would bind GATA-1/GATA-2 with high affinity as naked DNA, less than 1% are occupied in chromatin [18,19]. The mechanisms that endow motifs with the capacity to bind GATA factors in chromatin and why only certain occupied *cis*-elements confer nonredundant activity *in vivo* remain enigmatic.

Genomic maps of transcription factor occupancy and chromatin landscape are used to infer function. H3K4me1 and H3K27ac enrichments are interpreted to demarcate active enhancers [119▪▪]. Large chromosomal segments enriched in H3K27me3 and H3K4me3 (bivalent domains) are thought to poise loci for rapid activation during development [119▪▪]. p300 occupancy [120] is considered to pinpoint enhancers, whereas DNaseI hypersensitivity [121] and formaldehyde-assisted isolation of regulatory elements [122] score for accessibility. Although these approaches can lead to the discovery of putative *cis*-elements, predictions are tenuous without accompanying functional analysis at the endogenous locus. Another limitation is that mapping studies do not always utilize systems that recapitulate the relevant biology.

Traditional assays to evaluate *cis*-element function commonly rely on reporter gene measurements in transfected cells or transgene activity at ectopic chromatin sites. *Cis*-elements can elicit substantial activities within plasmids and at ectopic chromatin sites, which are irrelevant to endogenous locus function. The β-globin LCR consists of four DNaseI hypersensitivity sites approximately 15–50 kilo-bases upstream of the embryonic *ε*-globin and adult *β*major promoters, respectively [123,124], and is a pivotal determinant of β-like globin gene transcription at all developmental stages. The LCR confers position-independent and copy number-dependent expression of transgenes in transgenic mice, and targeted deletion of the hypersensitivity sites collectively in mice strongly reduces β-like globin expression at all developmental stages [125]. Many reports have documented impressive enhancer activities of individual hypersensitivity sites and subfragments thereof in transfection assays and transgenic mice. However, removing entire hypersensitivity sites of the LCR, which contain multiple *cis*-elements, only modestly (~10–30%) influence β-like globin expression [126]. Comparison of deletions of one or more of the hypersensitivity sites revealed that the individual hypersensitivity sites function additively to yield the powerful enhancer activity. This work, combined with numerous other studies, indicates that *in vitro* models of enhancer activity often do not recapitulate physiological function. The limitations of assessing *cis*-element function using historically accepted strategies are considerable.

The low frequency of GATA-1/GATA-2 occupancy of GATA motifs in chromatin, different permutations of GATA motifs (sequence variants, proximity to neighboring *cis*-elements, distinct chromatin attributes etc.), biologically critical functions of GATA-1 and GATA-2, unanswered questions about GATA switching and unresolved issues in epigenetics constitute a strong rationale for addressing how GATA motifs function at endogenous loci. An ideal system to explore this problem is the *Gata2* locus, given its five GATA switch sites, essential

function to control HSPC generation/function, and links to hematologic malignancies and vascular disorders.

Three GATA switch sites were deleted individually in mouse embryonic stem cells, and mutant mouse strains were generated. Deletion of the –1.8 site containing a conserved palindromic GATA motif revealed little to no role in activating *Gata2* expression, but it is essential for maintaining *Gata2* repression during erythroid maturation [127]. However, hematopoiesis is largely normal. Deletion of the –2.8 site, which contains multiple conserved GATA motifs, modestly reduces maximal *Gata2* expression, but does not influence *Gata2* repression upon erythroid maturation, nor does it significantly affect hematopoiesis [128]. Knockout of the intronic +9.5 site, which contains a conserved E-box–GATA composite element, is embryonic lethal at E14.5. $+9.5^{-/-}$ embryos have very few fetal liver HSPCs in E12.5 embryos, and *Gata2* expression is strongly reduced, consistent with the loss of *Gata2*-expressing cells [31▪,33▪] (Fig. 1). Although these sites share comparable GATA-1/GATA-2 occupancy, certain chromatin attributes and enhancer activity *in vitro*, their deletions yielded gross qualitative differences in activity.

Recent genome-editing innovations allow one to analyze *cis*-elements at endogenous loci in essentially any system [129▪▪,130▪▪]. The utility of this approach was highlighted in an application of transcription activator-like effector nucleases (TALENs) to delete a potential enhancer in intron 2 of *Bcl11a*, which encodes a fetal γ-globin repressor [131]. The deletion demonstrated the importance of the element for conferring *Bcl11a* expression in erythroid cells. Loss of the element downregulated BCL11A, which induced the γ-globin genes [132▪▪]. As γ-globin gene upregulation counteracts toxic effects of mutant β-globin in sickle cell disease, this study highlighted a potential therapeutic approach. The use of TALENs and zinc finger–nuclease fusions, along with the clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 system, to generate site-directed deletions is revolutionizing analyses of *cis*-element function at endogenous loci and their contribution to epigenetic mechanisms.

CIS-ELEMENT MECHANISMS UNDERLYING HEMATOPOIETIC PATHOPHYSIOLOGIES

Inherited and acquired hematologic disorders are often caused by mutations at loci essential for normal hematopoiesis. Although many mutations and polymorphisms in protein-coding regions have been described, less is known about variation in noncoding DNA motifs that influence disease susceptibility. Regulatory single nucleotide polymorphisms (SNPs) have been identified within a GATA motif upstream of the α-globin gene that causes α-thalassemia by interfering with activation of α-like globin genes [133] (Fig. 2). An intron 1 mutation disrupts a GATA motif in *ALAS2*, yielding X-linked sideroblastic anemia [134▪] (Fig. 2). SNPs within the *BCL11A* intronic enhancer impact hemoglobin levels and chromatin occupancy [132▪▪] (Fig. 2). Evidence for the role of *cis*-elements in suppressing malignant hematopoiesis emerged from analysis of patients with monocytopenia and mycobacteria infection (MonoMAC), an immunodeficiency associated with a predisposition for myelodysplastic syndrome and acute myeloid leukemia [135–138]. MonoMAC is caused

by heterozygous mutations in the DNA binding zinc finger of GATA-2 and appears to involve haplo-insufficiency [139]. One MonoMAC patient lacking coding region mutations harbors a heterozygous deletion of the E-box and five base pairs of the spacer of the +9.5 composite element (Fig. 1), and the phenotype of this patient is indistinguishable from those with GATA-2 zinc finger mutations [33▪]. Additional patients harbor point mutations in an ETS motif residing near the +9.5 composite element [139]. In a transfection context, the ETS motif was important for composite element enhancer activity [139]. It seems likely that further studies will reveal disruptions in many *cis*-elements that function nonredundantly to control hematopoiesis and/or hematopoietic cell function, and these alterations will underlie malignant and nonmalignant hematologic disorders.

Genome-wide association studies (GWASs) are increasingly identifying putative noncoding sequence variants linked to a predisposition for specific pathophysiologies. SNPs linked to predisposition for development of Hodgkin's lymphoma occur in genomic regions lacking known functions [140]. Seventy-five independent loci containing SNPs correlate with red cell phenotypes [141]. Although the majority of SNPs reside in noncoding regions, most GWASs have been disproportionately biased toward tabulating coding variants. Expanding these studies to identify additional noncoding variants that predispose for pathophysiological conditions will reveal novel disease-relevant loci that can be functionally validated using genome-editing technologies.

CONCLUSION

'Epigenetics' is often used to refer to chromatin modifying/remodeling mechanisms. Others argue that epigenetics should be restricted to scenarios involving unequivocal heritable transmission of traits without altered genetic content, which might or might not involve chromatin mechanisms. As *cis*-elements underlie chromatin-targeting specificity, and chromatin controls *cis*-element accessibility/function, disentangling genetic versus epigenetic contributions can be daunting. Sifting through abundant prospective *cis*-elements to identify those with nonredundant function is challenging. How many *cis*-elements resemble the +9.5 in controlling hematopoiesis nonredundantly? Can the thousands of GATA-1/GATA-2-occupied elements be segregated into functional versus nonfunctional elements via bioinformatics alone (Fig. 3)? How do critical *cis*-elements function in a context-dependent manner, for example, in distinct developmental stages? How important are combinatorial mechanisms in which merged *cis*-elements constitute entities with activities that cannot be predicted based on how the individual *cis*-elements function and factor occupancy patterns? To what extent do *cis*-element mutations, natural variation and alterations in chromatin mechanisms that control *cis*-element function underlie pathophysiologies and interindividual variation? Addressing these types of questions will yield important insights into red cell biology, normal and malignant hematopoiesis, and more broadly biological and genomic principles.

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KEY POINTS

- *Cis*-elements underlie the complex amalgamation of genetic and epigenetic mechanisms.
- GATA-1 and GATA-2 occupy a small fraction of their abundant *cis*-elements in a genome, and occupancy does not predict the functional output.
- Although predicting GATA motifs that function nonredundantly *in vivo* is not yet possible, multiple parameters collectively may have predictive value.
- Validation of *cis*-element function at endogenous loci is crucial, and novel genome-editing tools are revolutionizing such analyses.
- Mutational disruption of *cis*-elements underlies malignant and nonmalignant hematologic disorders, and single nucleotide polymorphisms within *cis*-elements can yield significant interindividual differences in hematologic parameters.

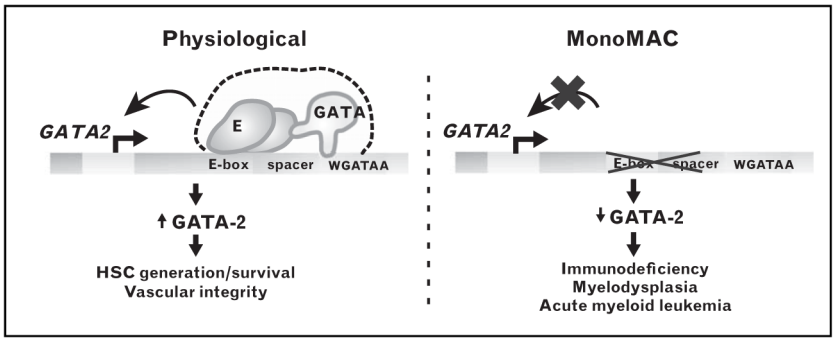

FIGURE 1.

Gata2 +9.5 *cis*-element. The *Gata2* intronic +9.5 GATA switch site, which contains an E-box–GATA motif composite element, functions nonredundantly to confer hematopoietic stem cell (HSC) generation in the aorta gonad mesonephros (AGM) region, the fetal liver hematopoietic stem/progenitor cell (HSPC) compartment and vascular integrity. Heterozygous mutation of the +9.5 element leads to monocytopenia and mycobacteria infection (MonoMAC) syndrome, with a phenotype indistinguishable from MonoMAC patients with *GATA2* zinc finger mutations. Adapted with permission from [33▪].

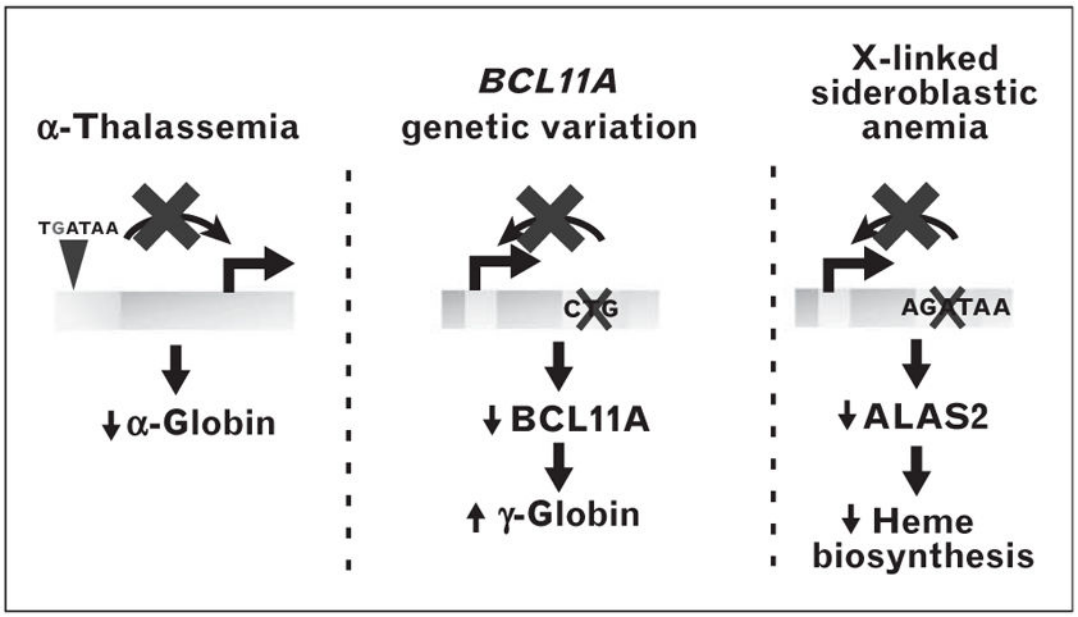

FIGURE 2.

Cis-element variation in normal and disease states. Mutational generation of a GATA motif interferes with α-globin transcription leading to α-thalassemia [133]. Natural variation in a GATA-1-binding region of the *BCL11A* locus as a determinant of γ-globin expression [132▪▪]. Mutational disruption of a GATA-1 motif reduces expression of *ALAS2*, which encodes a critical heme biosynthetic enzyme [134▪].

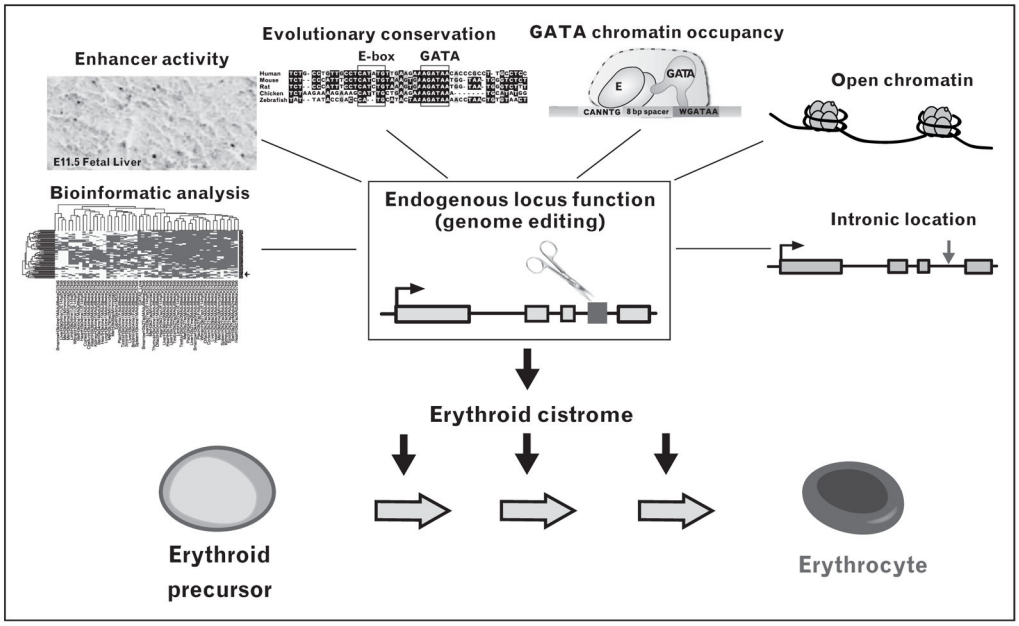

FIGURE 3.

Erythroid cistrome discovery strategy. Prospective *cis*-elements are prioritized based on multiple parameters and subjected to functional analysis by *cis*-element editing. GATA-1-occupied *cis*-elements functional at their endogenous loci are predicted to be important determinants of erythroid cell genesis and/or function.