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Complex developmental patterns of histone modifications associated with the human β -globin switch in primary cells

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Abstract

1) Objective: The regulation of the β -globin switch remains undetermined and understanding this mechanism has important benefits for clinical and basic science. Histone modifications regulate gene expression and this study determines the presence of three important histone modifications across the β -globin locus in erythroblasts with different β -like globin expression profiles. Understanding the chromatin associated with weak γ gene expression in bone marrow cells is an important objective with the goal of ultimately inducing postnatal expression of γ -globin to cure β -hemoglobinopathies.

2) Methods: These studies use uncultured primary fetal and bone marrow erythroblasts and human ES cell derived primitive-like erythroblasts. Chromatin immunoprecipitation (ChIP) with antibodies against modified histones reveals DNA associated with such histones. Precipitated DNA is quantitated by real time PCR for 40 sites across the locus.

3) Results: The distribution of histone modifications differs at each developmental stage. The most highly expressed genes at each stage are embedded within large domains of modifications associated with expression (H3ac and H3K4me2). Moderately expressed genes have H3ac and H3K4me2 in the immediate area around the gene. H3K9me2, a mark associated with gene suppression, is present at the ϵ and γ genes in bone marrow cells, suggesting active suppression of these genes.

4) Conclusion: This study reveals complex patterns of histone modifications associated with highly expressed, moderately expressed and unexpressed genes. Activation of γ postnatally will likely require extensive modification of the histones in a large domain around the γ genes.

Keywords

histone modification; beta globin; gene Expression; chromatin

I. Introduction

The β -globin switch is a process whereby expression of the β -like globin genes changes during development in a pattern that roughly correlates with shifts in sites of erythropoiesis and alterations in red cell morphology. The 5' most member of the human β -globin gene family,

ϵ -globin, is expressed predominantly by primitive red blood cells generated in the embryonic yolk sac. ϵ is separated from the $G\gamma$ and neighboring $A\gamma$ -globin genes by a 13.6 kb LINE repetitive element. The two γ -globin genes are primarily expressed by definitive red cells of the fetal liver and account for 80-86% of β -like expression with β making up the remainder. In adults, β accounts for over 95% of the β -like globin mRNA along with 2-4% δ and 1-3% γ [1].

Expression of γ -globins in adults is generally limited to F-cells, a small subpopulation of definitive red blood cells with 18-36% γ -globin containing hemoglobin [1]. F-cells are <10% of red cells in adults [2] and express fetal globins at a ratio different from that in the fetus: 2 $G\gamma$::3 $A\gamma$ in adults compared to 3 $G\gamma$::1 $A\gamma$ in fetuses [3].

The human γ to β -globin switch is of particular clinical interest because the effects of many β -hemoglobinopathies can be alleviated by expression of γ -globin. β -hemoglobinopathy patients with Hereditary Persistence of Fetal Hemoglobin (HPFH) have fewer episodes requiring clinical intervention than non-HPFH patients [4]. The activation of γ -globin to cure hemoglobinopathies is a long sought goal that has not yet been achieved because the regulation of β -globin switching is not yet fully understood. Insufficient γ -globin induction and toxicity limit the efficacy of current pharmacological treatments intended to induce γ .

Attempts to identify transcription factors involved in regulating the switch have isolated several which are important to gene expression and cellular identity [1,5], but none are sufficient to activate γ -globin expression to high levels. Studies of cis-acting regulatory elements in transgenic mice have shown that while the LCR is required for the normal high levels of β -like gene transcription, it is not necessary for low levels of transcription or switching of β -globin genes. These results suggest that the promoters or intergenic regions of individual genes are sufficient to regulate the globin switch [6,7].

Gene expression is influenced by chromatin structure as proposed by the histone code hypothesis. This theory posits that histone modifications form the elements of a "code" which is imposed, interpreted and enacted by trans-acting nuclear factors in order to regulate transcription [8,9]. Numerous studies supporting this show a frequent but not invariant correlation between specific histone modifications and transcription state(s). Methylation of K9 and K27 of histone H3 has been associated with transcriptionally repressed loci [10]. Acetylation of histones H3 and H4 and methylation of H3K4 is correlated with active transcription [11,12,13]. For most part, these studies describe differences between transcribed and untranscribed genes. There has been little focus on differences that relate to modulating levels of gene expression.

The relationship between different histone modifications and the β -globin switch has been studied in several vertebrate models. ChIP studies of chick cells have detailed a correlation between the transcription associated marks, H3K9ac, H3K14ac and H3K4me2 with expressed globin genes and a counter correlation of these marks with H3K9me2 [14]. In mice, β -globin like gene expression has been shown to correlate with H3ac and H4ac in an erythroleukemia cell line [15], adult bone marrow erythroid cells [16] and, yolk sac primitive and fetal liver definitive erythroid cells [15,17]. Similar findings were described in baboon erythroid cells [18]. The human erythroleukemia K562 cell line exhibits a strong correlation between H3K4me2, H3K3me3, H3K36me3 and H3K9me3 marks and β -globin like gene transcription [19]. H3ac are enriched at the γ -genes in primary fetal liver cells [20] and the δ - and β -globin genes of cultured primary human adult erythroid cells [20,21]. However, cultured adult erythroid cells express aberrantly high levels of γ ($\geq 25\%$ compared to 1-3% in primary bone marrow erythroblasts) [20] and are therefore not an optimal model to understand normal γ regulation in adults.

Despite considerable effort, current models do not recapitulate the essential features of the human switch. Human erythroid cell lines do not manifest the β -globin switch so they have limited utility in understanding switching. The two-stage murine switch does not fully model the 3 stage human switch since it lacks a fetal liver specific gene, therefore insights on switching in mice may not fully apply to humans, particularly in regards to the regulation of the γ -globins. Attempts to reiterate the human β -globin switch with transgenic mice containing the entire intact human β -globin gene cluster (hu β YAC) do not manifest the characteristic three-stage switch of humans. The baboon has a similar three-stage switch [22,23] but, unlike humans, high-level induction of γ -globin expression in adult baboons is readily achieved by a variety of means [24,25,26,27] suggesting there are significant differences between β -globin like regulation in baboons and humans.

To understand the details of chromatin changes associated with the normal β -globin switch, we have chosen to study uncultured primary human erythroblasts. Using approved protocols, erythroblasts from fetal liver and bone marrow were obtained and analyzed. Primary human primitive cells are very difficult to obtain; this was resolved by using a recently developed in vitro human embryonic stem cell culture system of generating primitive-like human erythroid cells [28,29].

II. Materials and Methods

Primary human erythroid cells

Primary human bone marrow and fetal liver samples were obtained using Institutional Review Board approved protocols. Fetal livers were supplied by Birth Defects Research Laboratory at University of Washington. Pure populations of nucleated erythroid cells were obtained by ficoll enrichment for nucleated cells followed by enrichment for glycophorin-A expressing cells using MACs antiglycophorin A magnetic beads (130-050-501, Milteny Biotec, Auburn, CA). Purified cells were scored visually by Wright-Geimsa stained cell smears and/or FACSScan to be $\geq 95\%$ nucleated erythroid. When sufficient numbers of cells were available, ChIPs were performed on samples of single individuals; when necessary, cells from several individuals (fetal livers) were pooled.

Human embryonic stem cell (huES) derived primitive-like erythroid cells were cultured as described in Qiu et al [28,29]. Briefly, huES cells were expanded and differentiated for 14 days on a human fetal liver hepatocyte (FHB) feeder layer followed by 14 or 21 days in liquid culture to generate primitive red cell like erythroid cells, hES14:14 or hES14:21 cells, respectively.

Chromatin immunoprecipitation

Chromatin immunoprecipitations were performed as described in the Upstate/Millipore protocol for anti-acetylated histone H3 (H3ac) antibody (cat.# 06-599) with modifications as described below. 1-3 million cells were cross-linked in PBS with 0.4% formaldehyde for 10 minutes at room temperature followed by lysis and sonication in lysis buffer [1% SDS, 10 mM EDTA, pH 8.0, 50 mM Tris-Cl, pH 8.1 and protease inhibitors (Roche cat.# 04 693 124 001; one mini tablet/10 ml lysis buffer)] to generate DNA fragments of approximately 200-500 bp lengths.

Cell lysates were diluted 1:6 with dilution buffer [0.01% SDS, 1.10% Triton X, 1.2mM EDTA, pH 8.0, 16.7 mM Tris-Cl, pH 8.1, 167 mM NaCl]. Lysate was precleared by shaking gently for 2-4 hours at 4°C with 50 μ l pre-immune sera and 100 μ l packed protein-A (or protein-G) agarose. Precleared lysate was briefly centrifuged to pellet the protein-A agarose, then divided into three to nine 700-900 μ l aliquots. 10 μ g of the appropriate antibody was added and the mixture was incubate overnight at 4°C, shaking gently.

The next day, 30 μ l packed protein-A or G agarose was added to each ChIP aliquot, and shaken gently for 2-4 hours at 4°C. Chromatin-antibody-protein-A/protein-G agarose complexes were washed once with 1 ml cold low salt wash buffer [0.1% SDS, 1% Triton X, 2 mM EDTA, pH 8.0, 20 mM Tris-Cl, pH 8.1, 150 mM NaCl]; once with high salt wash buffer [0.1% SDS, 1% Triton X, 2 mM EDTA, pH 8.0, 20 mM Tris-Cl, pH 8.1, 500 mM NaCl]; once with LiCl immune complex wash buffer [0.25 M LiCl, 1% IGEPAL-CA630, 1% deoxycholic acid, 1 mM EDTA, pH 8.0, 10 mM Tris-Cl, pH 8.1] and twice with TE buffer [10 mM Tris-Cl, pH 8.1, 1 mM EDTA, pH 8]. Antibody-chromatin complexes were reversed cross-linked by adding NaCl to 0.3 M final concentration and incubating at 65°C for 4-5 hours or overnight. The antibody bound chromatin DNA was purified by proteinase K digestion [add EDTA to 10 mM, Tris to 40 mM and Proteinase K to 80 μ g/ml; incubate at 55°C overnight] and a final passage through Qiagen PCR Purification columns (cat. # 28104). Concentration of chromatin immunoprecipitated DNA was estimated by PicoGreen (Molecular Probes) fluorescence with a Turner Biosystems fluorometer. The antibodies used in this study are: anti-acetylated histone H3 (H3ac), Upstate cat. # 06-599; anti-dimethyl lysine4 of histone H3 (H3K4me2), Upstate cat. # 07-030 and anti-dimethyl lysine 9 of histone H3 (H3K9me2), Upstate cat. # 07-441.

Primers

All primer sets amplify 80-200 bp of unique sequence within the human β -globin gene cluster. See Supplemental Table 1 for primer sequences.

Real time quantitative PCR

Quantitative PCRs (qPCR) were performed on chromatin immunoprecipitated DNA with the Biorad (Hercules, CA-USA) iQ SybrGreen 2x qPCR Mix (cat. # 170-8882) in an MJ Research Opticon Thermocycler. Approximately one nanogram of immunoprecipitated DNA was used for each reaction. Data is presented as fold enrichment over negative control. The validity of each chromatin immunoprecipitation was determined by amplification of control sites. For the antibodies used in this study, an immunoprecipitation was acceptable if there was a three fold or greater difference between the expressed (hemogen) and unexpressed (pseudo-amphiphysin or necdin) controls. Samples which did not meet these criteria were removed from the data set. Error bars represent the variability between the average of multiple ChIPs performed on at least 2 independent samples.

To control for the efficiency of individual chromatin immunoprecipitation assays, qPCRs against a site of active transcription, hemogen, and sites that are transcriptionally silent, pseudo-amphiphysin and/or necdin, were included. Hemogen encodes a highly transcribed hematopoietic nuclear factor [30] on chromosome 9. Pseudo-amphiphysin is a non-transcribed pseudogene distally linked to the beta-globin gene cluster located 85 kb upstream of epsilon in the odorant receptor gene cluster 5' of the beta-globin locus. The functional amphiphysin gene on chromosome 7 encodes a neuron-specific protein found on synaptic vesicles [31]. The neuron specific necdin gene is located on chromosome 15 [32,33,34].

The primary cells used for this study were collected from unrelated individuals. To assess variation among individuals and replication(s) of the assay, we determined the reproducibility of ChIP results from biological (variation between individuals) and technical (variation within repeated assays of the same sample) replicates. Technical replicates of H3ac ChIP assays of one individual were highly reproducible (see Supplemental fig. 1) and the distribution of this mark in six individuals (three samples) are comparable (see Supplemental fig. 2), demonstrating consistency between donors. Due to the consistency and reproducibility of distribution patterns of modified histones within and between samples, the number of technical and biological replicates were reduced to 1-3. The actual number of sites surveyed and technical

replicates performed was dependent on the amount of DNA recovered from each ChIP assay, which varied with the sample size.

II. Results

The presence of histone marks across the β -globin gene cluster were assessed by ChIP at forty sites between the HS5 core of the LCR to 200 bp past β -globin (figure 1A). The coding regions of ϵ and the two γ -globin genes are each represented by two sites in the 5' region of the respective genes. $G\gamma$ and $A\gamma$ -globin genes are very similar and cannot be readily differentiated by PCR, thus the results of γ -globin assays are equally attributed to the two genes. The coding regions of δ and β -globin are represented by a single site located towards the 3' end of their respective genes. The remaining sites are distributed throughout the locus. The numerous repetitive elements in the locus were not directly assayed although some adjoining sequences were. For the purposes of discussion, enrichment is defined as 2 fold or greater over background (2 fold over negative control). A histone modification is considered depleted when its level is less than two fold the level of the negative control.

H3ac and H3K4me2 are associated with transcribed β -like globin genes

$G\gamma$ and $A\gamma$ -globins are the predominant members of the β -globin gene cluster transcribed by fetal liver erythroblasts in addition to modest levels of β -globin. As can be seen in Fig. 1B, the γ -globin genes are enriched for H3ac and H3K4me2 in fetal erythroblasts where the highest enrichment is within and between the genes themselves. The moderate levels of these marks at the γ -globin promoters may reflect the absence of histones due to displacement by RNA polymerase II initiation complex. H3ac/H3K4me2 enrichment encompasses a domain that reaches beyond the γ -globin genes (see shaded regions in Fig. 2A). The 5' region of this domain, from the $G\gamma$ promoter to the large 13.6 kb LINE element, is enriched for both marks. The 3' boundary of the fetal γ -globin domain is less distinct, H3ac enrichment ends in pseudo- β and the H3K4me2 domain extends to just downstream of pseudo- β . In addition, the promoter and coding region of β -globin is also enriched in H3ac and H3K4me2 (Fig. 2A), consistent with its low-level pancellular expression [35,1]. Although there are no reports of δ -globin expression in fetal erythroblasts, both marks are present upstream and at the promoter of δ . ϵ is not expressed in fetal cells and the entire surrounding region, from the LCR to the large LINE element, is depleted of H3ac, although modestly enriched for H3K4me2 histone marks (Fig. 1B).

The correlation between transcriptionally permissive histone marks in extended domains and gene expression is maintained in bone marrow. H3ac and H3K4me2 are enriched in an extensive domain of positive marks that spans the region from 5' of δ to 3' of β (Fig. 1C, shaded regions), similar to the γ -globin domain in fetal liver. Apart from DNase hypersensitive sites in the LCR, there are no other regions of the locus enriched for positive marks in bone marrow cells. This pattern of histone modifications reflects the high levels of pancellular β -globin expression, low levels of pancellular δ -globin expression, and low levels of heterocellular γ -globin expression exhibited by adult erythroblasts [1]. F-cell expression of the γ -globins genes in adults is not reflected in positive histone marks.

Because primary human primitive erythroblasts are difficult to obtain, huES cell derived primitive-like erythroid cells were used to model embryonic ϵ -globin expression. The maturation of cultured huES cells along an erythroid lineage in this system is highly synchronous: hES14:14 cells are primarily basophilic erythroblasts and hES14:21 cells are mostly orthochromatic erythroblasts. ϵ expression by hES14:14 cells is 40% and γ expression is 60% of total β -like globin. With an additional week in culture, hES14:21 cells advance to expressing 90% γ -globins and 10% ϵ at the RNA level [28,29]. ChIP results of hES14:14 and hES14:21 cells show that H3ac is enriched in an extended domain surrounding ϵ -globin from

HS1 of the LCR to the large LINE element (Fig. 2A and 2B). This pattern is analogous to the extended chromatin domains in fetal liver and bone marrow erythroblasts around the most highly expressed β -like genes.

In primitive-like cells, there is consistent enrichment of H3ac in sites within and between the γ -globin genes. These regions are modestly enriched in H3K4me2 in hES14:21 cells while the promoters and γ -globin genes themselves are depleted of H3K4me2 in hES14:14 cells (compare Fig. 2A to 2B, right sides). This is the only instance in which the promoters and coding regions of clearly expressed genes are not enriched for both positive marks. There is sporadic enrichment around δ and β -globin for H3K4me2, but not for H3ac despite the lack of significant transcription in these cells. In addition, similar to ϵ -globin, the distal 5' flanking sites of G γ (downstream of the 13.6 KB LINE repeat) are enriched for H3K4me2 in both hES14:14 and hES14:21 cells but these sites are only enriched for H3ac in hES14:14 cells (compare Fig. 2A to 2B).

The LCR hypersensitive sites are enriched in H3ac and H3K4me2 in human erythroid cells

H3ac and H3K4me2 levels are variably enriched at most LCR DNase hypersensitive sites with values ranging from minimal H3ac enrichment at HS2 in adult erythroblasts (Fig. 1) to greater than sixteen fold levels of H3K4me2 at HS4 in hES14:21 cells (Fig. 2). The exceptions are the low levels of H3K4me2 at HS2 in bone marrow erythroid cells (Fig. 1) and at HS1 and HS2 in hES14:14 cells (Fig. 2). It is not clear whether these lower levels signal the lack of histone modifications or if they represent a state of histone depletion; DNase hypersensitive sites are known to be depleted of nucleosomes. These results suggest that HS1-HS5 are actively engaged in supporting stage appropriate high level expression of all members of the β -globin gene family.

H3K9me2 enrichment correlates with the absence of transcription at the β -globin locus

H3K9me2 is generally considered a mark of transcriptionally silent chromatin and is found particularly enriched in heterochromatin and repetitive elements [10,36]. In the β -globin gene cluster of definitive erythroblasts, the highest levels of H3K9me2 are found at sites flanking the 13.6 kb LINE element between ϵ and G γ -globin. H3K9me2 is also enriched at globin genes during erythroid stages where they are not expressed. This includes ϵ , δ and β -globin in fetal erythroblasts (Fig. 3A) and ϵ -globin and promoters of the γ -globins in bone marrow erythroblasts (Fig. 3B). It is apparent that low level pancellular expression of β -globin by fetal liver cells does not preclude the enrichment of H3K9me2 at this gene although it does not form an extended chromatin domain. Similarly, H3K9me2 does not form an extended domain around the γ genes in bone marrow erythroblasts. H3K9me2 is highly enriched in an extended region surrounding ϵ -globin in fetal and bone marrow erythroblasts, starting past HS1 of the LCR and extending through the gene to the LINE element. This is the only extended chromatin domain uniformly marked with the H3K9me2 repressive mark and it correlates with the lack of detectable ϵ -globin expression in definitive cells.

Discussion

Stage specific chromatin domains surrounding the β -like genes reflect their expression state

High level pancellular expression of β -like globin genes in primary human erythroid cells is associated with 10+ kb chromatin domains of H3ac and H3K4me2 enrichment over regions which encompass and are significantly larger than the genes themselves. Similar domains of H3K9me2 enrichment correlate with fully silent β -like genes. These domains of histone modifications shift over the course of the β -globin switch to accommodate developmental changes in globin gene expression.

The H3K9me2 mark (associated with silent genes and heterochromatin) is found at the promoters of the γ -globins in bone marrow erythroblasts and across most of the broad ϵ -globin domain in fetal liver and bone marrow erythroblasts (Fig. 3). A number of Set domain family proteins catalyze the methylation of H3K9, creating binding sites for a variety of chromo domain proteins to mediate transcriptional [37,38]. The presence of H3K9me2 suggests that this mechanism may be actively restricting expression, more robustly at ϵ than at γ -globin, and suggests that the lack of γ expression in bone marrow is due to such a suppressive mechanism.

The spatial coincidence of H3K9me2 with the H3ac/H3K4me2 positive marks at δ and β -globins in fetal liver cells is an unusual instance in which positive and negative histone modifications occur together. The combination of repressive and permissive marks may function as a molecular rheostat to limit β -globin expression to the modest levels observed in fetal liver erythroblasts.

Insights into γ -gene induction in bone marrow erythroblasts

These studies illuminate the chromatin impediments to pharmacological activation of γ in patients with β -hemoglobinopathies. To gain significant clinical benefit, γ -globin expression must be increased many fold over normal adult levels [4], ideally by pancellular induction of γ -globin expression. High level expression of β -globin like genes is associated with the presence of positive and absence of negative histone marks in broad domains surrounding and including the expressed genes. This pattern of histone modifications is also likely required to achieve effective induction of therapeutically effective levels of γ -globin expression. Figure 1C shows that the γ -globin genes of bone marrow erythroblasts completely lack positive histone marks. But in adults, only the γ -globin genes of the small γ expressing F-cell fraction are likely marked by positive histone modifications. In a bulk biochemical assay like ChIP(s), the γ genes would seem fully depleted of permissive marks (Fig. 1C). Therefore, the vast majority of γ -globin alleles appear not poised for expression due to the absence of positive marks and the presence of negative modifications at the gene promoters. This suggests that pharmacological induction of γ -globin in adults requires an extensive remodeling of γ -globin chromatin to relieve H3K9me2 repression and establish transcriptionally permissive chromatin structure.

The chromatin structure differences between ϵ and γ in bone marrow reflects the ability to induce these genes either genetically (through HPFH) or pharmacologically. ϵ -globin is marked by a broad domain of negative H3K9me2 modifications, whereas the γ -globins are only sporadically marked with H3K9me2. As a reflection of this, expression of ϵ is not reported in HPFH and ϵ is completely uninducible by pharmacological means. It is possible that the lack of a broad H3K9me2 domain around the γ -globins in bone marrow erythroblasts permits the therapeutically useful sporadic γ induction.

Extensive chromatin domains at all highly expressed genes have not been observed in other β -globin like models

Baboons are, evolutionarily, the closest animal model whose β -globin switch has been extensively analyzed [22,23]. Like humans, levels of γ -globin are low in normal adult baboons, however, these animals express high pancellular levels of γ -globin after treatment with dacogen or decitabine [26,27], drugs which are only weakly inductive of γ -globin in humans [4,5] or when anemia is induced by blood loss [39]. The γ -globin promoters of adult baboon erythroblasts are marked by intermediate levels of H3Ac and H3K4me2, between the negative neccin control and the promoter of the highly expressed β -globin [18]. This is not seen at the human γ -globins where permissive marks are present at levels equal to that of the unexpressed negative control. These differences in chromatin structure could explain why γ -globin expression is more difficult to induce in humans than in baboons.

The tissue specific domain pattern of transcriptionally positive histone modifications we observe in the human β -globin gene cluster is unusual [340,41] but not unique. A similar pattern has been described by Bernstein et al in the human and mouse HOXA cluster [42]. The tightly regulated timing and tissue specificity of both globin and HOX gene expression suggests that the shared epigenetic domain organization is a transcriptional regulatory mechanism that may be part of other multigenic loci.

Transcriptionally permissive γ -globin epigenotype never occurs in isolation

High level fetal specific expression of γ -globins in higher primates has been postulated a late evolutionary development. Phylogenetic and expression analysis of humans and other mammals suggests that prior to recruitment for fetal expression, the γ -globins were active during embryonic erythropoiesis [43,44,45,46]. The intermediate nature of γ gene expression is reflected in the lack of enrichment of positive marks, H3ac and H3K4me2, at the γ -globins in the absence of similar marks at either ϵ or β -globins. In huES cell derived erythroid cells, ϵ and γ -globins are enriched in H3ac and H3K4me2 (Fig. 3). In fetal erythroblasts, modest enrichment of these marks at β -globin is concurrent with high levels at the γ -globins (Fig. 1). It is notable that fetal cells express higher levels of $G\gamma$ than $A\gamma$ and this relationship is reversed in adults. γ -globin expression in adult F-cells may be mediated by the stochastic spread of permissive chromatin from the δ/β domain towards the γ -globins. The proximity of $A\gamma$ to the active δ/β domain increases its likelihood to be affected by the spread of transcriptionally permissive chromatin giving $A\gamma$ a transcriptional advantage over $G\gamma$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1. Extended domains of H3ac and H3K4me2 around expressed genes in primary human erythroid cells

(A) Scaled map of the human β -globin gene cluster. Ovals denote DNase hypersensitive sites of the LCR; thick black bars, are genes as labeled; thin gray bars are repetitive elements. (B, C) Distribution of H3ac (left) and H3K4me2 (right) in fetal liver (B) and bone marrow (C) erythroblasts. Results are plotted on X-axis over physical map. Black data bars represent test sites; gray data bars represent control sites as marked by (+, hemogen) and (-, pseudo-amphyphysin/neccdin). Shaded regions denote domains marked by H3ac or H3K4me2. Dotted line marks minimal enrichment. Data is expressed as fold difference over negative control. Standard error of the mean indicated by error bars.

Figure 2. H3ac and H3K4me2 epigenotype of huES derived primitive-like cells
H3ac (left) and H3K4me2 (right) distribution in (A) hES14:14 and (B) hES14:21 primitive-like erythroid like cells. Results are presented as in Fig. 1.

Figure 3. Extended domains of H3K9me2 mark the silent ϵ gene in uncultured primary human cells

H3K9me2 distribution in (A) fetal liver and (B) bone marrow erythroblasts. Results are presented as fold difference over the expressed control, hemogen. The results for hemogen are (-) and the results for pseudo-amphyphysin/neccidin are (+).