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Combinatorial Assembly of Developmental Stage-Specific Enhancers Controls Gene Expression Programs during Human Erythropoiesis

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SUMMARY

Gene-distal enhancers are critical for tissue-specific gene expression, but their genomic determinants within a specific lineage at different stages of development are unknown. Here we profile chromatin state maps, transcription factor occupancy, and gene expression profiles during human erythroid development at fetal and adult stages. Comparative analyses of human erythropoiesis identify developmental stage-specific enhancers as primary determinants of stage-specific gene expression programs. We find that erythroid master regulators GATA1 and TAL1 act cooperatively within active enhancers but confer little predictive value for stage specificity. Instead, a set of stage-specific coregulators collaborates with master regulators and contributes to differential gene expression. We further identify and validate IRF2, IRF6, and MYB as effectors of an adult-stage expression program. Thus, the combinatorial assembly of lineage-specific master regulators and transcriptional coregulators within developmental stage-specific enhancers determines gene expression programs and temporal regulation of transcriptional networks in a mammalian genome.

INTRODUCTION

Erythropoiesis in mammals occurs in three waves consisting of primitive progenitors in the yolk sac, definitive precursors in the fetal liver and later in the postnatal bone marrow (McGrath and Palis, 2008; Orkin and Zon, 2008). Several transcription factors (TFs), such as GATA1 and TAL1 (or SCL), are essential for erythroid development and are recognized as the erythroid "master" regulators (Cantor and Orkin, 2002). These lineage-specifying master regulators, together with other transcription factors and cofactors, act within complexes on chromatin, establish transcriptional networks, and orchestrate differentiation (Kim and Bresnick, 2007). Master regulators of different lineages often cross-antagonize each other's activity during lineage specification (Graf and Enver, 2009). However, it is less clear how master regulators control programs at different stages of development within the same cell lineage.

A gene regulatory network consists of *trans*-acting regulators and *cis*-acting elements within core promoters and gene-distal enhancers whose interaction with each other control tissue- and developmental stage-specific programs (Bulger and Groudine, 2011). Genome-wide studies suggest that enhancers and promoters exhibit distinct chromatin "signatures." The characteristic signature for enhancers consists of monomethylation of histone H3 lysine 4 (H3K4me1), acetylation of histones (H3K9ac and H3K27ac), and binding of the acetyltransferase p300 (Heintzman et al., 2007; Koch et al., 2007; Visel et al., 2009). Studies comparing various lineages indicate that enhancers are associated with highly cell-type-specific histone modifications and strongly correlate to global cell-type-specific programs (Blow et al., 2010; Creyghton et al., 2010; Ernst et al., 2011; Ghisletti et al., 2010; Heintzman et al., 2009; Mikkelsen et al., 2010; Rada-Iglesias et al., 2011). Hence, it has been suggested that enhancers are the primary determinants of cell-type specificity in gene expression. However, the majority of studies have employed cells from very different lineages and/or immortalized cell lines. It remains unknown the degree of overlap between cells within a specific lineage at different stages of differentiation or similar cells at different stages of development.

We reasoned that comparative profiling of closely related cell types corresponding to distinct developmental stages should delineate regulatory networks directly related to the associated gene expression programs. Classification of the *trans*- and *cis*-regulatory elements that are either shared or stage specific should clarify their relative importance and prioritize functional candidates. We have focused on an ex vivo maturation system for human fetal and adult erythropoiesis. Primary hematopoietic

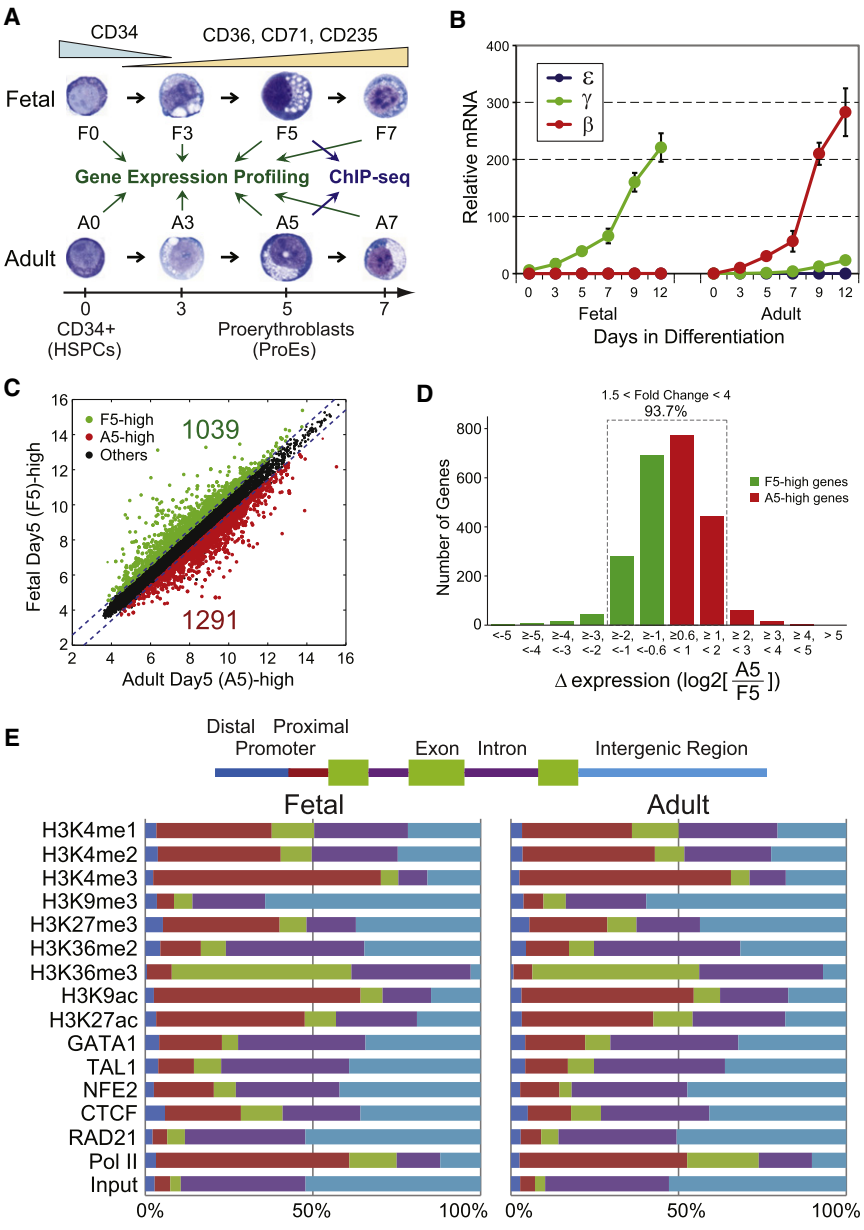

Figure 1. Comparative Genomic Analyses of Human Erythropoiesis

(A) Fetal and adult CD34+ HSPCs were differentiated into ProEs ex vivo. Cells at matched stages of differentiation were collected for gene expression profiling and ChIP-seq analyses.

(B) Expression of human embryonic (ε), fetal (γ), and adult (β) globin mRNAs. Results are means ± SD of at least three independent experiments.

(C) Scatterplots of gene expression profiling between fetal and adult ProEs (F5 and A5). Dashed blue lines indicate the 1.5-fold differential expression cutoff to define the F5-high (increased expression in F5 relative to A5 ProEs) or A5-high (increased expression in A5 relative to F5 ProEs) genes. The numbers of differentially expressed genes are indicated.

(D) Numbers of differentially expressed genes conditional on changes in expression levels between A5 and F5 ProEs.

(E) The genome-wide distribution of the profiled histone marks and TFs. Total numbers of enriched regions in distal promoters (blue), proximal promoters (red), exons (green), introns (purple), and intergenic regions (light blue) are identified (Experimental Procedures). The graph shows the fraction of enriched regions for each histone mark and TF in fetal and adult ProEs, respectively.

See also Figures S1 and S2, and Tables S1 and S2.

stem/progenitor cells (HSPCs) can be propagated and induced for erythroid differentiation with a set of defined cytokines ex vivo. This system has been widely used in molecular analysis of erythropoiesis (Migliaccio et al., 2009).

Here, we report the comparative investigation of genome-wide chromatin state maps, TF occupancy, and gene expression profiles from developing red cell precursors at two developmental stages. Distal enhancers, not promoters, are marked with highly stage-specific histone modifications and DNase I hypersensitivity, strongly correlate to stage-specific gene expression changes, and are functionally active in a stage-specific manner. Master regulators GATA1 and TAL1 act cooperatively within active enhancers but have little predictive value for stage-specific enhancer activity. In contrast, a set of stage-specific cofactors and signaling pathways collaborate with these regulators and account for the stage specificity. Two such cofactors, the interferon regulatory factors (IRFs) 2 and 6, are essential for activation of adult erythroid programs through cooperation with master regulators and cohesin-mediator complexes at distal enhancers.

RESULTS

Ex Vivo Maturation of Primary Human Fetal and Adult Erythroid Progenitors

We employed a serum-free two-phase liquid culture system to expand and differentiate primary fetal or adult-stage human CD34+ HSPCs ex vivo (Sankaran et al., 2008). In this experimental context, highly enriched populations of stage-matched, differentiating, primary proerythroblasts (ProEs) were generated (Figure 1A). We selected four time points (day 0, CD34+ HSPCs; days 3, 5, and 7, differentiating ProEs) that represented similar stages of differentiation (Figure 1A; see Figures S1A, and S1B available online). Adult CD34+ HSPC-derived ProEs expressed predominantly adult hemoglobin (β-globin). Conversely, fetal ProEs expressed largely fetal hemoglobin (γ-globin) (Figure 1B), indicating that the ex vivo system faithfully recapitulates stage specificity.

Comparative Profiling of Gene Expression, Chromatin Signatures, and Transcription Factor Occupancy

We determined the mRNA expression profiles in fetal and adult HSPCs and differentiating ProEs by Affymetrix microarray