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Developmental changes in DNA methylation and covalent histone modifications of chromatin associated with the ε-, γ-, and β-globin gene promoters in *Papio anubis*

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Abstract

The baboon is a suitable and relevant animal model to study the mechanism of human globin gene switching. This investigation addresses the role of DNA methylation and histone coding in globin gene switching in the baboon, *Papio anubis*. Bisulfite sequencing and chromatin immunoprecipitation studies were performed in erythroid cells purified from fetuses of varying gestational ages and from adult bone marrow to analyze the manner that changes in DNA methylation of the ε-, γ-, and β-globin promoters and association of ac-H3, ac-H4, H3-dimeK4, H3-dimeK36, and H3-dimeK79 with the ε-, γ-, and β-globin promoters occur during development. Changes in DNA methylation of the ε- and γ-globin gene promoters during transitional stages of globin gene switching were consistent with the stochastic model of methylation and a role of DNA methylation in gene silencing. Enrichment of ac-H3, ac-H4, and pol II at the promoters of developmentally active genes was observed, while the pattern of distribution of H3-dimeK4 and H3-dimeK79 suggests that these modifications are found near both currently and formerly active promoters. Enrichment of H3-dimeK36 at the silenced ε-globin gene promoter was observed. These studies demonstrate that coordinated epigenetic modifications in the chromatin structure of the β-like globin gene promoters accompany the highly regulated changes in expression patterns of these genes during development.

Keywords: Hemoglobin switching; DNA methylation; Chromatin

Introduction

The five β-like globin genes, ε–γ1–γ2–δ–β, are located within the 70 kb β-globin gene complex on chromosome 11 in humans. The ε-globin gene is the major β-like globin gene expressed in primitive, yolk-sac-derived red blood cells. During human development, a shift in the site of erythropoiesis from yolk sac to liver coincides with ε-globin silencing and predominant expression of γ-globin. Later in gestation, a second shift in the site of erythropoiesis from liver to bone marrow coincides with increased β-globin expression and decreased γ-globin expression. Completion of the switch from γ- to β-globin expression occurs postnatally. Although it has been intensively investigated, the mechanism responsible for hemoglobin switching is unknown [1].

Globin gene structure and the pattern of expression during development are conserved in Old World simian primates, but not in prosimians or other species [2]. Simian primates are therefore suitable animal models to study the mechanism of human hemoglobin switching. Changing patterns of DNA methylation and combinatorial covalent histone modifications, referred to as the histone code, modulate chromatin structure and impose a new level of regulatory information upon the primary DNA sequence [3–5]. A detailed description of changes in DNA methylation and chromatin structure of the β-globin gene locus during development would increase understanding of the molecular mechanism of hemoglobin switching and could lead to the design of new rational molecular therapies for the reactivation of fetal hemoglobin (HbF) in patients with β-thalassemia and sickle cell disease.

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Previous analysis of globin gene methylation by Southern blot in combination with methylation-sensitive restriction enzymes was limited to CpG sites contained within the recognition sequences of the restriction enzymes used. In these experiments, a general anti-correlation between globin gene expression and DNA methylation during human development was observed [6,7]. The ability of DNA methyltransferase inhibitors to reactivate HbF to high levels in adult baboons demonstrated the importance of DNA methylation in γ-globin gene silencing [8]. During the evolutionary transition from prosimians to simians, the γ-globin gene attained a fetal developmental stage-specific expression pattern that coincided with acquisition of a set of CpG dinucleotides in the γ-globin gene promoter [9]. Recruitment of the γ-globin gene to fetal stage expression required the development of a new mechanism to silence its expression during the fetal to adult transition, and methylation of these CpG dinucleotides may be important in this process.

Globin gene switching in chickens also involves changes in globin gene methylation. The development of bisulfite sequencing technology, allowing examination of the methylation state of potentially every CpG site within a gene [10], identified extensive methylation of a CpG rich region within the proximal promoter region of the chicken embryonic ρ-globin gene in primary adult erythrocytes that was unmethylated in primitive embryonic cells [11]. Methylation of these sequences inhibited transcription in vitro and promoted formation of a complex containing MBD2 [12].

The role of covalent histone modifications in globin gene switching has been investigated in chickens and mice [13]. The chicken β-globin gene locus was enriched in acetylated histones H3 (ac-H3) and H4 (ac-H4) and histone H3-meK4, while surrounding chromatin was enriched in H3-meK9 [14,15]. In other studies, it was observed that H3-dimeK4 was broadly distributed throughout the locus, while H3-trimeK4 was associated with the promoters of developmentally active genes [16]. Transcriptionally active globin gene promoters in mouse fetal liver were enriched in acetylated histones and the inactive promoters were hypoacetylated, while in yolk sac, both active and inactive promoters were hyperacetylated. In the adult stage, the mouse embryonic Ey and βH1 genes are located in a 30 kb chromatin subdomain characterized by low levels of histone acetylation and H3-dimeK4 [17–19]. Enrichment of H3-meK79 in chromatin surrounding developmentally active globin genes was also observed in the mouse and therefore this covalent histone modification was suggested to be involved with the mechanism of globin gene switching [20]. Studies in human progenitors and K562 cells suggest that the globin gene locus is enriched in acetylated histones at early stages of hematopoietic differentiation [21,22]. In this investigation, we describe changes in DNA methylation of the ε- and γ-globin gene promoters and changes in covalent histone modifications of chromatin associated with the ε-, γ-, and β-globin gene promoters in purified erythroid cells during development of the simian primate, *P. anubis*, to gain more insight into the molecular mechanism responsible for globin gene switching in primates.

Materials and methods

Baboons

Blood, liver, and bone marrow tissues were obtained from fetal baboons, *P. anubis*, produced by timed matings at the University of Illinois Biologic Resources Laboratory. Fetuses were euthanized by injection with euthobarb prior to the removal of tissue. Bone marrow cells from the hips of 3- to 4-year-old phlebotomized baboons were obtained by aspiration. The baboons were acutely bled to a hematocrit of 20 by the daily removal of 16–18% of the packed cell volume. The hematocrit was maintained during the remainder of the treatment. Bone marrow aspiration was performed 10 days after the first phlebotomy. All procedures were approved by the IACUC of the University of Illinois at Chicago.

Cell purification

Low-density mononuclear cells were enriched from bone marrow aspirates by Percoll gradient sedimentation. Cell suspensions were prepared from fetal livers by passing finely minced tissue through a wire screen.

Erythroid cells were purified from fetal blood and liver cells and from low-density mononuclear cells from fetal and adult bone marrow by magnetic column separation using an antibody to baboon red blood cells (Pharmingen) in combination with anti-mouse IgG1 microbeads (Miltenyi). Purity of cell preparations was evaluated by microscopic examination of Wright-stained cytospin preparations.

Bisulfite sequence analysis

DNA was isolated from purified erythroid cells using Qiagen kits and bisulfite modification performed [23]. The ε-, γ-, and β-globin gene promoters were amplified by two rounds of PCR with semi-nested primers. PCR products were cloned in the pCR4 vector (Invitrogen). For the γ-globin promoter, the initial round of PCR was performed using primers BG1 (TATGGTGGGAGAAGAAATTAGTTAAAGG) and BG2 (AATAACCTTATCCTCCTCTATAAAATAACC), and the second round using primers BG2 and BG5 (GGTTGGTTAGTTTTGTTTTGATTAATAG). For the ε-globin promoter, the initial round of PCR was performed using primers BE1 (GGAAATTTGTGTTGTAGATAGATGAGG) and BE2 (CAATACTTACCTACCCAAAACTTCACC), while the second round was performed using primers BE1 and BE3 (CAATAAAATACACCATAATACCAAACC). For the β-globin promoter, the first round of PCR was performed using primers MBR341 (TGAGATAGAGTTTTTATTTATTTTGTTTTG) and MBR342 (CCTCCCTCTAAAACATATCTCTTAACC), while the second round was performed with MBR341 and MBR343 (TATCTCTTAACCCCATACCATCAATAC). Sequence analysis of at least 10 random clones from each sample was performed using an ABI Prism 300 genetic analyzer at the University of Illinois DNA Sequencing Facility.