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# SATB1 regulates β-like globin genes through matrix related nuclear relocation of the cluster

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ABSTRACT

The nuclear location and relocation of genes play crucial regulatory roles in gene expression. SATB1, a MAR-binding protein, has been found to regulate β-like globin genes through chromatin remodeling. In this study, we generated K562 cells over-expressing wild-type or nuclear matrix targeting sequences (NMTS)-deficient SATB1 and found that like wild-type SATB1, NMTS-deficient SATB1 induces out loop of β-globin cluster from its chromosome territory (CT), while it is unable to associate the cluster with the nuclear matrix as wild-type SATB1 does and had no regulatory functions to the β-globin cluster. Besides, our data showed that the transacting factor occupancies and chromatin modifications at β-globin cluster were differentially affected by wild-type and NMTS-deficient SATB1. These results indicate that SATB1 regulates β-like globin genes at the nuclear level interlaced with chromatin and DNA level, and emphasize the nuclear matrix binding activity of SATB1 to its regulatory function.



## Introduction

Accumulating evidence showed that the nucleus is well organized, where each chromosome occupies distinct nuclear position and forms discrete entity termed chromosome territory (CT) [1]. Previous studies suggested that the localizations of genes relative to their CTs are regulated in correspondence with their activities [2,3]. The human β-globin gene cluster is a paradigm for further studying the mechanisms of gene regulation at nuclear level. This cluster locates at the short arm of chromosome 11 and consists of five developmental stage specific genes—embryonic (ε), fetal (Gγ, Aγ) and adult (δ, β) globins, and a powerful far upstream locus control region (LCR) which comprises five DNase I hypersensitive sites (HSs). In erythroid cells, the β-globin locus is looped out from its CT even before transcriptional induction [4] indicating that the extrusion should be considered as a poised state prior to activation rather than a consequence of transcription.

In nuclei, chromatin fibers are organized into loops (5–200 kb) by attaching the structural constraints at their bases—the nuclear matrix. Besides being a skeleton, nuclear matrix is also considered as platform for regulatory factors. Recent studies suggested that matrix/scaffold attachment regions (MARs/SARs) bind with matrix in a discriminatory manner and the selecting process depends on the structural and functional requirements of the cell [5]. SATB1 (special AT-rich binding protein 1), a MAR-binding protein, has been considered to be a new type of gene regulator. It provides docking sites for specialized DNA sequences and enzymes to regulate gene expression over long distances [6–8]. Amino acids 224–278 of SATB1 make up of a nuclear matrix targeting sequence (NMTS) and are important to the formation of the unique birdcage-like structure of SATB1 [9,10].

Previous study showed that over-expression of SATB1 in K562 cells increased ε-globin gene expression and decreased γ-globin gene expression by remodeling chromatin states [11]. Our recent work suggested that SATB1 is the critical component of an "inter-MAR association", which contributes to the transcriptionally active looping events in human β-globin gene cluster [12]. In this report, we further investigated the mechanism through which SATB1 regulated the expression of the cluster. Functional significance of its nuclear matrix targeting character of SATB1 was further addressed by observing the effects of wild-type and the NMTS-deficient-SATB1 on the expression and nuclear positioning of β-globoin gene cluster.

## Materials and methods

*Cell culture.* Human erythroleukemia K562 and HeLa cells (PUMC Culture Collection) were cultured in RPMI 1640/10% FBS. Induction of K562 cells were carried out under 50 μM hemin for 72 h.

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*Plasmid construction.* The SATB1 expression vector-pEGFP/SATB1 was constructed by amplifying SATB1 cDNA from pE-CHAT1146 via PCR [13], ligating it into pEGFP-N2 (Clontech, Palo Alto, CA). The NMTS-deletion SATB1 expression vector-pEGFP/mSATB1 was constructed via recombinant PCR. Briefly, two fragments were separately amplified from the SATB1 cDNA with primer pair1 and pair2 (see Supplementary Material). The NMTS was deleted in the second step PCR with primer A and D using the mixture of products from the two first step PCRs as a template. The product of the second step was ligated into pEGFP-N2 to give rise to pEGFP/mSATB1. The sequence accuracy of the two plasmids was confirmed by DNA sequencing. Stably transfected cell lines were generated through introducing pEGFP/SATB1 or pEGFP/mSATB1 into K562 cells by Lipofectamin 2000 and selected with geneticin.

*Halo preparation.* Nuclear halos were prepared essentially as described [5].

*DNA FISH.* Two dimensional FISH was performed as described [14]. Human chromosome 11 paint was from Cambio (Cat. No. 1066-11B-02–1). BAC probe for human β-globin cluster was nick translated by DIG-Nick translation Mix (Roche, Germany), using BAC186D7 as the template [15]. Chromosome paints were used according to manufacturers' recommendations with 100–200 ng of BAC probes. Positions of BAC probes with respect to CTs and nuclear matrix were scored in Adobe Photoshop. The looping frequency for each cell line is equal to [extruded β-globin cluster/total β-globin cluster scored]. The matrix-associated frequency was equal to [associated β-globin cluster/total β-globin cluster scored]. $\chi^2$ test was used for statistically analysis and the threshold of statistical significance was $P < 0.05$.

*Chromatin immunoprecipitation.* Chromatin extracts were prepared as described previously [16] with minor modifications in our lab [17]. Antibodies against RNA Polymerase II (Pol II) and GATA1 were purchased from Santa Cruz biotechnology, Inc. (USA). Antibodies against acetylated H3 and H4 were obtained from Upstate biotechnology (Lake Placid, NY). GAPDH was used as internal control.

## Results

To investigate the influence of SATB1 on globin gene expression and further explore the related regulatory mechanism, we generated stably transfected K562 cells with wild-type or NMTS-deficient SATB1 overexpression called SATB1/K562 or mSATB1/K562 cells, respectively (Fig. S1 A–D). The expression of the exogenous wild-type or NMTS-deficient SATB1 was confirmed with RT-PCR analysis (Fig. S1E).

### *Wild-type but not NMTS-deficient SATB1 over-expression has regulatory effects on β-like globin genes expression*

K562 cells express predominantly ε- and γ-globin, and trace level of β-globin mRNA (Fig. S2). RT-PCR analysis indicated that, in SATB1/K562 cells, the changes of ε-, γ-, and β-globin transcripts levels were in consistent with previous report [11]. On the contrary, in mSATB1/K562 cells, the mRNA levels of these genes were close to those in the mock and untransfected K562 cells (Fig. S2). These results suggested that NMTS-deficient SATB1 has no regulatory effects on β-like globin genes expression as wild-type SATB1 has.

### *Both wild-type and NMTS-deficient SATB1 induce looping of the β-globin locus*

It is reported that activated β-globin locus prefers to extrude from its CT [4]. Firstly, to confirm the relationship between moving out and activation of β-globin locus, we applied FISH to observe the positioning of β-globin cluster relative to its CT in either the uninduced K562 cells or hemin induced K562 cells. The β-globin locus did not show significant relocation (only 13.8%) away from its CT in uninduced K562 cells (Fig. 1A and F); while in hemin induced K562 cells, the percentage increased to 37% (Fig. 1B and F). This result confirmed that the extruding of β-globin locus is in association with its activation.

Next, we performed FISH to determine the effects of SATB1 on β-globin cluster relocation. About 30% β-globin alleles located outside of the CT No.11 in SATB1/K562 cells (Fig. 1C and F). This result implied that SATB1 may play roles in relocating β-globin locus away from its CT. Unexpectedly, in mSATB1/K562 cells, although NMTS-deficient SATB1 had no effects on β-like globin genes expression, the percentage of relocated β-globin locus (32%) had no significant difference from that in SATB1/K562 cells (Fig. 1D and F). The relocation was not induced by the vector itself as the frequency of looping in the EGFP/K562 cells (12%) was very similar to that in the uninduced K562 cells (Fig. 1E and F). These data demonstrated that both wild-type and NMTS-deficient SATB1 induce looping of the β-globin locus.

### *Wild-type not NMTS-deficient SATB1 anchors β-globin cluster on nuclear matrix*

To distinguish the extrusion of β-globin locus induced by wild-type and NMTS-deficient SATB1, nuclear halos were prepared from K562, SATB1/K562 and mSATB1/K562 cells for FISH assays with BAC probes covering β-globin locus. The percentage of nuclear matrix associated β-globin clusters in SATB1/K562 cells (95%, Fig. 2B) is similar to that in K562 cells (93%, Fig. 2A). In contrast, in mSATB1/K562 cells, the percentage is only 71% (Fig. 2C). As the NMTS-deficient SATB1 lost part of the nuclear matrix binding character, these results suggested that NMTS was involved in the function as anchors.

### *SATB1 affects binding statuses of Pol II and GATA1 in a matrix dependent manner*

To further characterize the SATB1-associated nuclear relocation of β-globin locus, we detected the binding patterns of Pol II and GATA1 on the pivot *cis*-elements within β-globin locus in K562, SATB1/K562 and mSATB1/K562 cell lines via ChIP assays. As necdin is silent in the erythroid cell lines, the binding situations of these factors on necdin promoter were used as negative control.

Our results showed that the binding frequencies of Pol II increased on HS2 and ε-gene promoter but decreased on γ-globin gene promoter in SATB1/K562 cells compared to K562 cells. While there exhibited no significant differences between mSATB1/K562 cells and K562 cells of Pol II binding status on these detected sites (Fig. 3A). These properties were consistent with the changes at transcriptional levels of β-like globin genes.

GATA1 is a repressor of ε-globin gene [18]. Since no significant change of GATA1 expression level was found when wild-type SATB1 was over-expressed in previous study [11], we further examined binding statuses of GATA1 on three reported binding sites within the 5′ flanking region of ε- globin gene (+3, −165, −213) [19]. It demonstrated that the binding frequencies of GATA1 on all these three sites were decreased in SATB1/K562 cells and were similar in mSATB1/K562 cells compared to K562 cells (Fig. 3B).

The differences between the binding patterns of the Pol II and GATA1 on β-globin locus in SATB1/K562 and mSATB1/K562 cells implied that SATB1 affects binding statuses of them in a matrix dependent manner.