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BP1 motif in the human β -globin promoter affects β -globin expression during embryonic/fetal erythropoiesis in transgenic mice bearing the human β -globin gene

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Abstract

The binding of the transcription factor BP1 (β protein 1) to its site on the promoter of the adult β -globin gene has a silencing effect on β -globin transcription *in vitro*. To better understand the mechanism of BP1's negative regulation of β -globin expression, we developed transgenic mice bearing a human β -globin locus-containing cosmid. Specifically, we introduced a mutated BP1 binding site (mtBP1) into the promoter of the β -globin gene sequence of this cosmid construct. In the mtBP1 mice, we detected a more than 20-fold increase in human β -globin expression in the yolk sac-derived blood at E10.5, a 3-fold increase in fetal livers at E13.5, and an approximately 1.4-fold increase in adult reticulocytes compared with control mice bearing the human β -globin gene with the wild-type BP1 binding site sequence (wtBP1). Our *in vivo* observations support the contention that the BP1 binding site of the β -globin promoter plays an important role in the regulation of transcription of the adult β -globin gene.

Keywords

Human β -globin promoter; BP1 binding site; Human β -globin expression; Transcription factor; Transgenic mice

Introduction

During erythroid cell development, globin gene expression within the β -globin cluster is achieved through a precise balance of negative and positive regulation. Current hypotheses about the possible mechanisms for negative regulation include competition between genes for interaction with the locus control region (LCR) [1] and autonomous gene silencing involving sequences located in the proximal and distal promoters [2–4]. BP1 (β protein 1) plays an important role in β -globin gene regulation, because binding of BP1 to its site on the promoter of the adult β -globin gene has a silencing effect on β -globin transcription *in vitro* [5–9]. The

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adult β -globin gene includes two upstream silencer regions (silencer I and II), and BP1 binds to both of these regions [5,8]. In our earlier studies of BP1 function in K562 cells, an erythroleukemia cell line with an embryonic-fetal phenotype, we have shown that introduction of multiple mutations in two BP1 binding sites enhances β -globin promoter activity [6]. Inhibition of BP1 expression also has been demonstrated to increase levels of β -globin mRNA in K562 cells [9]. To better understand effects of the negative regulation of β -globin expression at the level of the whole organism, we have developed a transgenic mouse expressing the human β -globin gene mutated at the BP1 binding site. Because the silencer II element does not interfere with high mobility group (HMG) protein binding sites as silencer I does [7], we mutated the silencer II BP1 binding site in a cosmid construct that we subsequently used to create transgenic mice. To study human β -globin gene expression during the transgenic mouse embryo development, we analyzed embryonic and fetal blood, and adult reticulocytes of the transgenic mice. We have also studied the role of BP1 in β -globin expression in human and mouse cell lines. In mouse cells, we analyzed effects of Dlx4 expression, which is the most probable mouse ortholog of BP1 [8], on human β -globin expression.

Materials

Cell cultures and transfection

K562 and U937 cells (ATCC, Manassas, VA) were grown in RPMI 1640 culture medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin. Murine erythroleukemia cell line (MEL) were grown in IMEM culture medium supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 μ g/ml gentamicin. Cells were regularly subcultured to maintain exponential growth. Transfection of cells was performed using an electroporation apparatus (Nucleofector System, Amaxa, Koeln, Germany). Cells (2×10^6) were transiently transfected with 5 μ g of plasmid DNA (enhanced green fluorescent protein-reporter constructs) using the manufacturer's recommended Nucleofector kit (Amaxa).

Enhanced green fluorescent protein (EGFP)-reporter constructs

EGFP-reporter constructs were prepared as described previously [9]. Briefly, a 690-bp DNA fragment of the adult human β -globin regulatory region, from -640 to +50 relative to the cap site (+1), was amplified by the polymerase chain reaction (PCR) from a 35.4-kb cosmid containing the β -globin gene locus. The 690-bp PCR product, containing the β -globin promoter and an upstream regulatory region including silencers I and II, was ligated to the *HindIII*/*BamHI* cloning sites of the multi-site promoterless vector pEGFP-1 (Clontech, Mountain View, CA) to drive transcription of an EGFP-reporter gene. Mutations in the BP1 binding site (silencer II, positions -294 to -302) were introduced as described earlier [9]. The wild-type silencer II sequence, 5'-TTCAATATG-3', was mutated to 5'-TTGCTCGAC-3', and was confirmed by automated DNA sequencing.

Transgenic mice

A 13-kb fragment of the 35.4-kb cosmid construct (μ LCR $A_{\gamma\psi\beta\delta\beta}$) was released from the cosmid construct by digestion with *SalI* and ligated to the *SalI* site of the multi-sited pBluescript II KS+ and pUC18 vectors, creating pBKS (#1) and pUC18 (#2) constructs, respectively. An *Apal/XbaI* fragment (β -globin gene locus coordinates: 60882–65421) was excised from construct #1 and cloned into another pBluescript II KS+ vector at its *Apal/XbaI* sites to create construct #3. An *SphI/SnaBI* PCR fragment of the promoter region of the β -globin gene (61519–61869) containing the mutated BP1 binding site was inserted into construct #3 that had been previously digested with *SphI/SnaBI*. An *Apal/SnaBI* fragment (60882–61869) containing the mutated BP1 binding site was excised from construct #3 and inserted into construct #2 that had been previously digested with *Apal/SnaBI*. A 13-kb fragment of construct

#2 containing the mutated BP1 binding site was digested with *SalI* and coupled with a 22-kb fragment of the 35.4-kb cosmid construct previously digested with *SalI*. Mutations in the BP1 binding site (silencer II) were reconfirmed by automated DNA sequencing. The experimental constructs are diagrammed in Fig. 1A.

The μ LCR $\Lambda_{\gamma\psi\beta\delta\beta}$ fragment (with mutated BP1 sequence) was excised from the 35.4-kb cosmid construct by digestion with *NotI* and *KpnI* for microinjection. The purified DNA fragment was injected into fertilized mouse eggs (FVB/N) and then transferred to pseudopregnant foster mothers (CB6 F₁) to develop mutant transgenic mice bearing the β -globin gene locus with a mutated BP1 binding site (subsequently referred to as mtBP1 mice). The control transgenic mice bearing the μ LCR $\Lambda_{\gamma\psi\beta\delta\beta}$ fragment with the wild-type BP1 binding site sequence (subsequently referred to as wtBP1 mice) were developed as described previously [10]. Founder animals were identified by Southern blotting of DNA extracted from tail biopsies. Southern blots were probed with human γ - and/or human β -globin probes. Founder animals were crossed to FVB/N mice for propagation and developmental studies. Genotyping of F₁ and F₂ transgenic mice was performed by PCR to amplify a 493-bp fragment of the human γ -globin gene (Fig. 1B). Copy number was determined by comparing transgenic mouse genomic DNA to K562 DNA in real-time PCR experiments. To study human β -globin gene expression in embryos, staged pregnancies were interrupted on embryonic day 10.5 (E10.5) and 13.5 (E13.5) of development.

Reverse transcription-PCR and quantitative real-time PCR assay

Total RNA was isolated from human or mouse cells using RNA STAT-60 total RNA/mRNA isolation reagent (Tel-Test, Inc., Friendswood, TX) according to the manufacturer's protocol and quantitated by absorbance at 260 nm. Each 50 μ l of reverse-transcription (RT) reaction (Ready-to Go RT-PCR beads, Amersham Biosciences, Piscataway, NJ) contained 1 μ g total RNA, 250 ng oligo-dT, 200 mM concentrations of each dNTP, 200 U of reverse transcriptase and ribonuclease inhibitor, and 2.0 U of *Taq* DNA polymerase. The reaction mixtures were incubated at 42°C for 30 min, then at 95°C for 5 min. Following the addition of 100 ng of a gene-specific primer set to each tube, the RT reaction mixtures were then subjected to PCR.

Quantitative real-time PCR assays of transcripts were performed using gene-specific double-labeled fluorescent probes in a 7700 Sequence Detector (PE Applied Biosystems, Foster City, CA) as previously reported [9].

RT- and TaqMan PCR oligos are shown in Table 1.

All probes were designed to span exon junctions in the fully processed message. Standard curves were based on accurately determined dilutions of reference plasmids containing human γ - or β -globin or mouse α -globin cDNA as a template. Plasmid dilutions covered a dynamic range of five logarithmic orders or greater. The quantity of globin mRNA per μ g of total RNA was calculated using constant threshold levels and standard curves.

The reference-plasmids used in TaqMan PCR experiments were assembled by TOPO TA Cloning using pCRII-TOPO vector (Invitrogen, Carlsbad, CA). The cDNA fragments encompassing the human β - and γ -globins (338- and 397-bp fragments, respectively) and mouse α -globin (431-bp fragment) were produced by RT-PCR from hematopoietic cells of mtBP1 transgenic mice. The cDNA fragments were coupled to the pCRII-TOPO cloning site and sequenced to confirm the expression of human β - and γ -globins in the transgenic mice.

Fluorescence intensity analysis of digital images

Fluorescence intensity analysis of digital images was performed as described earlier [9]. Fluorescence was measured by capturing digital fluorescent images (1200 $\times$ 1600 pixels) from

transfected MEL, K562, or U937 cells growing in 12-well plates, using a digital RT-KE 3-shot color F-mount camera (Opelco, Dulles, VA) mounted on an inverted fluorescence microscope (Olympus IX51) equipped with a 100-W mercury lamp. Off-line image analysis was performed using Image-Pro Plus software, version 5.0 (Media Cybernetics, Des Moines, IA). Mean pixel intensity (8-bit pixel depth) was calculated from each cell; background fluorescence was subtracted from all values. Statistical significance was determined by Student's *t*-test.

Results

Transgenic mice with cosmid gene constructs

Three transgenic mouse lines (designated as A, B, and C) were generated with the cosmid construct containing the mutated BP1 binding site sequence (mtBP1). Real-time PCR analysis showed that these lines contained between two and eight copies of the cosmid. After correction for copy number, the mean human β -globin gene mRNA levels in adult reticulocytes from mtBP1 mice and wtBP1 mice were 102% and 81% relative to the level of mouse α -globin mRNA, respectively (Table 2).

TaqMan PCR analysis of β -globin expression

The mRNA levels of human β -globin in mtBP1 mice were higher at all stages of erythroid cell development when compared with wtBP1 mice (Table 2). In wtBP1 mice, γ -globin was predominantly expressed in yolk sac and fetal liver, while β -globin mRNA was detected in fetal liver and adult erythroid cells. In contrast, mtBP1 mice expressed human β -globin mRNA in all three tissues examined (Table 2). At E10.5, we detected a more than 20-fold increase in β -globin expression in yolk sac-derived blood from mtBP1 mice (3.8%, $n=9$) compared with levels in wtBP1 mice (0.17%, $n=6$, $p<0.001$, Table 2, Fig. 2A). At E13.5, mRNA levels of human β -globin in fetal livers of mtBP1 mice (62%, $n=9$) were approximately three-fold higher than in wtBP1 mice (21%, $n=6$, $p<0.005$, Table 2, Fig. 2B). These data indicate that mtBP1 transgenic mice have drastically higher human β -globin transcript levels in E10.5-13.5 blood cells as compared with control wtBP1 transgenic mice.

TaqMan PCR analysis of *Dlx4* expression in mouse erythroid cells

Dlx4 is the most probable murine ortholog of BP1 [8]. The mRNAs of BP1 (GI:11141506) and *Dlx4* (GI:75677402) are highly homologous (80%) and their homeobox sequences encode almost identical protein DNA-binding domains. We found that *Dlx4* mRNA was highly expressed in the yolk sac-derived embryonic blood of E10.5 mouse embryos, and its expression in erythroid cells gradually decreased during embryo development (Fig. 3). We detected very low levels of *Dlx4* transcripts in adult bone marrow, and no expression of *Dlx4* in adult reticulocytes (Fig. 3). In addition, we observed that *Dlx4* mRNA levels in whole 11-day embryos were lower than in yolk sac-derived embryonic blood (data not shown).

Comparative *in vitro* analysis of activities of β -globin promoters of two cosmid constructs

In previous studies, we analyzed the *in vitro* activities of β -globin promoters of two cosmid constructs in K562 cells, a human embryonic erythroleukemia cell line [9]. Specifically, we introduced β -globin promoters with wtBP1 and mtBP1 binding sites into a promoterless EGFP plasmid. In those studies, we detected a three-fold increase in EGFP expression in cells transfected with the EGFP-reporter construct containing the β -globin promoter with the mtBP1 binding site as compared with the wtBP1 β -globin promoter. In the current study, we transfected a murine erythroleukemia cell line (MEL) with these two EGFP constructs (Fig. 4A–F). We performed quantitative fluorescence intensity analysis of digital images of MEL cells transfected with the mutated BP1 binding site construct (Fig. 4A–C) and with the wild-type construct (Fig. 4D–F). Quantitative fluorescence intensity analysis of digital images did

not indicate a significant difference in the promoter activities of wtBP1 (31.3 ± 0.7 grey shadows/pixel, $n=411$) and mtBP1 (32.2 ± 0.8 grey shadows/pixel, $n=464$) EGFP constructs in MEL cells. Analysis of *Dlx4* expression in the MEL cells did not detect *Dlx4* mRNA in that cell line, but showed high levels of mouse β -major mRNA (Fig. 4G). We also transfected K562 cells with these two EGFP constructs (Fig. 5A–F), and performed quantitative fluorescence intensity analysis of digital images of K562 cells transfected with the mutated BP1 binding site construct (Fig. 5A–C) and with the wild-type construct (Fig. 5D–F). In K562 cells, the activity of the β -globin promoter was approximately three-fold higher for the mtBP1 construct than that for the wtBP1 construct (45.1 ± 0.7 grey shadows/pixel, $n=677$ versus 15.8 ± 0.2 grey shadows/pixel, $n=558$, $p<0.001$). These data are consistent with our earlier results [9]. In addition, we transfected a U937 human cell line with these two EGFP constructs (Fig. 6A–F). The U937 cell line is a myeloid cell line with monocytic phenotype that expresses BP1 (Fig. 7). Quantitative fluorescence intensity analysis of digital images detected an approximately 1.5-fold increase in the promoter activity of the mtBP1 EGFP construct as compared with the wtBP1 construct in U937 cells (28.8 ± 0.6 grey shadows/pixel, $n=316$ versus 19.6 ± 0.4 grey shadows/pixel, $n=204$, $p<0.001$). The BP1 expression in U937 cells resulted in a difference in the β -globin promoter's activities in the two EGFP constructs, with higher expression of EGFP for the mtBP1 construct as compared to the wtBP1 construct.

Discussion

The major finding of this study is that transgenic mice bearing the human β -globin gene with a mutated BP1 binding site (mtBP1) have significantly higher human β -globin transcript levels in E10.5–13.5 blood cells as compared with control transgenic mice bearing the human β -globin gene with a wild-type BP1 binding site (wtBP1). The differences in human β -globin expression between the mutant and control mice may be due to the fact that mRNA of murine ortholog of BP1, *Dlx4*, is also predominantly expressed in embryonic blood at E10.5.

Specifically, we have made several observations. First, mutating the DNA sequence of silencer II (in the BP1 binding site of the human β -globin promoter, Fig. 1A) increased β -globin expression. Expression of the human β -globin mRNA was significantly increased (more than 20-fold) in yolk sac-derived embryonic blood of mtBP1 transgenic mice at E10.5 as compared with expression in control (wtBP1) transgenic mice. We also detected a three-fold elevation of mRNA levels of human β -globin in E13.5 fetal liver of mtBP1 mice as compared to wtBP1 mice, and found that human β -globin expression in adult reticulocytes of mtBP1 mice was up to 1.4-fold higher than in adult reticulocytes of wtBP1 mice. Second, the expression of mouse *Dlx4* in E10.5 yolk sac-derived embryonic blood was higher than its expression in E13.5 fetal liver or adult erythroid cells. Our PCR data indicated that yolk sac blood was a primary site of *Dlx4* expression, because the total RNA isolated from whole 11-day embryos had less *Dlx4* mRNA expression than that from embryonic blood.

Finally, we performed *in vitro* analysis of β -globin promoter activity of EGFP-reporter constructs in MEL, U937, and K562 cells. When MEL cells were transfected with an EGFP-reporter construct driven by a β -globin promoter with either a wtBP1 or a mtBP1 binding site, elevated activities on a similar scale were observed. In contrast, K562 cells transfected with an EGFP-reporter construct containing the β -globin promoter with the mtBP1 binding site demonstrated an increase in EGFP expression as compared with cells transfected with an EGFP-reporter construct containing the wtBP1 binding site β -globin promoter. We found that mouse *Dlx4* was not expressed in the MEL cell line, which can explain the difference in results obtained using MEL and K562 cells. While the MEL cell line did not express *Dlx4*, it expressed mouse β -major mRNA at high levels. It is very likely that the absence of *Dlx4* and, therefore, the inability of the negative regulator of β -globin transcription to bind its site, allowed not only higher mouse endogenous β -major expression but also human β -globin-EGFP-reporter

construct expression. In this *in vitro* set of experiments, we also transfected the BP1-expressing U937 human cell line with the two constructs, and found higher expression of the β -globin promoter's activities in EGFP constructs with the mtBP1 construct when compared with the wtBP1 construct.

Previous experiments from our and other laboratories have shown that BP1 represses the β -globin gene [5–9]. The BP1 protein binding site in both silencers has been determined by DNase I footprint analysis [5] and its consensus sequence, (A/T)T(A/C)(A/T)ATATG, has been deduced [8]. It has previously been shown that BP1 is an isoform of human DLX4 [8]. The DLX4 gene belongs to the Distal-less (DLX) family of homeobox genes, which encode transcription factors that are essential for early development. The Distal-less family of genes comprises at least six different members, DLX1-DLX6 [11]. Murine Dlx4 shares 88% DNA sequence homology with BP1 thus raising the possibility that the murine Dlx4 actually corresponds to BP1 [8]. The mRNAs of BP1 and murine Dlx4 are highly homologous (80% homology), and their homeobox sequences encode almost identical protein DNA-binding domains. The differences in the mRNA expression of human β -globin between mtBP1 and wtBP1 mice may be due to the fact that murine Dlx4 mRNA is also predominantly expressed in embryonic blood at E10.5. We do not know whether Dlx4 binds to the silencer II sequence directly, or through a partner protein. To our knowledge, the mechanism has not yet been established. Future experiments would be needed to confirm if mouse Dlx4 also binds to the silencer II element as BP1 does.

A possible mechanism for the regulation of human adult β -globin was described in our earlier publications [9,12]. Briefly, BP1 binding to its silencer regions induces HMG-mediated DNA bending that impairs the binding of positive regulators, such as EKLF and GATA-1 (Fig. 8). When a DNA silencing motif is mutated, BP1 cannot bind to its target sequence. This destabilizes the DNA-protein and protein-protein interactions of the entire silencing complex, thereby allowing the positive regulators access to the promoter to induce gene expression (Fig. 8). To this end, BP1 might work in concert with other transcription factors involved in the regulation of human adult β -globin, but as of yet have not been identified.

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Figure 1.

Wild-type and mutated BP1 binding site sequences in the promoter of human β -globin gene located in a 35.4-kb cosmid construct of the β -globin gene locus. (A) Transgenic mice bearing the control 35.4-kb cosmid construct (μ LCR $A\gamma\psi\beta\delta\beta$) with wild-type BP1 binding site sequence (Wt) were generated as described previously [10], and transgenic mice bearing the mutated BP1 binding site sequence (MtBP1) in the β -globin promoter were generated as described in Materials. (B) Founder transgenic mice were identified by Southern blot analysis using the human β -globin gene as probe, then genotyping of F₁ and F₂ transgenic mice was performed by PCR to amplify a 493-bp fragment of the human γ -globin gene. The figure represents the results of one genotyping experiment. (C) The cDNA fragments encompassing the human β -globin (338-bp fragment) were produced by RT-PCR from hematopoietic cells of wild-type (Norm) mice and F₁ mtBP1 transgenic (Tg) mice of line A and B (line C is not shown). The cDNA fragments of all transgenic mtBP1 mice were sequenced to confirm expression of human β -globin as described in Materials.

Figure 2.

Expression of human β -globin in E10.5 yolk sac-derived embryonic blood (A and C) and E13.5 fetal liver (B) of mtBP1 and wtBP1 transgenic mice. The bars represent the mean of all lines shown in Table 2. (C) Expression of human β -globin and γ -globin in E10.5 yolk sac-derived embryonic blood of mtBP1 (mt, line C) and wtBP1 (wt, line B) transgenic mice. One μ g of total RNA of transgenic mouse yolk sac blood was subjected to TaqMan PCR using primers and probe designed for evaluation of transcripts of human β -globin or γ -globin as described in Materials. Mouse α -globin transcript expression was also examined as a control. The final products of the TaqMan PCR reaction were separated on a 1% agarose gel. The resultant product sizes were as follows: β -globin, 181 bp; α -globin, 190 bp; γ -globin, 219 bp. The figure displays results from one representative set of reactions.

Figure 3.

Expression of mouse *Dlx4* mRNA in E10.5 yolk sac-derived blood, E13.5 fetal liver, adult bone marrow, and adult reticulocytes. One μ g of total RNA of mouse yolk sac blood, fetal liver blood, adult bone marrow (BM), and adult reticulocytes was subjected to TaqMan PCR using primers and probe designed for evaluation of transcripts of mouse *Dlx4* as described in Materials. Mouse β -actin transcript expression was also examined as a control. The final products of the TaqMan PCR reaction were separated on a 1% agarose gel. The resultant product sizes were as follows: *Dlx4*, 148 bp; β -actin, 182 bp. Gel images were collected and quantitated using ImagePro Plus 5.0 software as described earlier [9]. Expression of *Dlx4* at

E10.5 was elevated up to 3.5-fold of that observed at E13.5 and up to 40-fold of that observed in adult bone marrow. The figure displays results from one representative set of reactions.

Figure 4.

Expression of human β -globin in MEL cells in the lack of expression of Dlx4. (A–F) Digital fluorescence microscopy analysis of EGFP expression in MEL cells driven by the wild-type (Wt) or mutated (MtBP1) β -globin promoter. Quantitative fluorescence intensity analyses of digital images of MEL cells transfected with the mtBP1 construct (A–C) or the wtBP1 construct (D–F). A and D are fluorescent images; B and E are surface plots of fluorescence intensity of images shown in A and D, respectively. C and F are fluorescence histograms of images shown in A and D, respectively. The figure displays the results from one representative experiment. (G) Expression of mouse Dlx4, β -major, and β -actin mRNA in MEL cells. One μ g of total RNA from MEL cells was subjected to RT-PCR using primers designed for evaluation of transcripts of mouse Dlx4, β -major, or β -actin as described in Materials. The final products of the RT-PCR reaction were separated on a 1% agarose gel. The resultant product sizes were as follows: Dlx4, 380 bp; β -major, 280 bp; mouse β -actin, 410 bp.)

Figure 5.

Digital fluorescence microscopy analysis of EGFP expression in K562 cells driven by the wild-type (Wt) or mutated (MtBP1) β -globin promoter. Quantitative fluorescence intensity analyses of digital images of K562 cells transfected with the mtBP1 construct (A–C) or with the wtBP1 construct (D–F). A and D are fluorescent images; B and E are surface plots of fluorescence intensity in images shown in A and D, respectively. C and F are fluorescence histograms of images shown in A and D, respectively. The figure displays the results from one representative experiment. Note pixel intensity redistribution with increase in the number of pixels with higher mean fluorescence value displayed by cells transfected with the mtBP1 construct (C).

Figure 6.

Digital fluorescence microscopy analysis of EGFP expression in U937 cells driven by the wild-type (Wt) or mutated (MtBP1) β -globin promoter. Quantitative fluorescence intensity analyses of digital images of U937 cells transfected with the mtBP1 construct (A–C) or with the wtBP1 construct (D–F). A and D are fluorescent images; B and E are surface plots of fluorescence intensity of images shown in A and D, respectively. Note higher fluorescence intensity in cells transfected with MtBP1 construct (B) as compared with cells transfected with Wt (E). C and F are fluorescence histograms of images shown in A and D, respectively. The figure displays the results from one representative experiment. Note pixel intensity redistribution with increase

in the number of pixels with higher mean fluorescence value displayed by cells transfected with the mtBP1 construct (C).

Figure 7.

Expression of BP1 mRNA in K562 and U937 cells. One μ g of total RNA from K562 and U937 cells was subjected to RT-PCR as described in Materials. Human β -actin transcript expression was also examined as a control. The final products of the RT-PCR reaction were separated on a 1% agarose gel. The resultant product sizes were as follows: BP1, 224 bp; human β -actin, 626 bp. The figure displays results from one representative set of reactions.

Embryonic-Fetal Hematopoietic Cells

Adult Hematopoietic Cells

Figure 8. Model of the potential mechanism for the regulation of β -globin gene expression. LCR, Locus Control Region; HMG, High Mobility Group proteins.

Table 1

Oligos used for RT- and TaqMan PCR analysis

Gene	RT-PCR primer	Product size (bp)
BP1	5'-GCTGAAAGAGGCTCAGAGAGA-3' forward	
	5'-AGGTCTGGGAAGACAGCTTTG-3' reverse	224
β -globin	5'-CTTTAGTGATGGCCTGGCTC-3' forward	
	5'-GGCAGAATCCAGATGCTCAAG-3' reverse	338
γ -globin	5'-GAACGTCTGAGATTATCAAT-3' forward	
	5'-GAAATGGATTGCCAAAACGG-3' reverse	397
α -globin	5'-CTGAATATGGAGCTGAAGCC-3' forward	
	5'-GACCAAGAGGTACAGGTGCA-3' reverse	431
Gene	TaqMan PCR primers and probes	Product size (bp)
β -globin	5'-CTTTAGTGATGGCCTGGCTC-3' forward	
	5'-ATAGGCAGCCTGCACTGGTG-3' reverse	
	5'-CAGGCTCCTGGGCAACGTGCTGGTCTG-3' probe	181
γ -globin	5'-GGCAACCTGTCCTCTGCCTC-3' forward	
	5'-GAAATGGATTGCCAAAACGG-3' reverse	
	5'-CAAGCTCCTGGGAAATGTGCTGGTG-3' probe	219
α -globin	5'-CTGAATATGGAGCTGAAGCC-3' forward	
	5'-TCGCTCAGAGCAGACAAG-3' reverse	
	5'-GAAAGGATGTTTGCTAGCTTCCCCACC-3' probe	190
Dlx4	5'-CATCTCAGCCCTTTACAG-3' forward	
	5'-AGCTGCAGGCTAGAGTAG-3' reverse	
	5'-CAGAATCAGAGAAGCTGGCACTGTCTC-3' probe	148
β -actin	5'-ATATCGCTGCGCTGGTCG-3' forward	
	5'-GATACCTCTCTTGCTCTG-3' reverse	
	5'-CACCAGGGTGTGATGGTGGGAATG-3' probe	182

Expression of human β - and γ -globins in transgenic mouse lines

Construct	Line	Mean human γ -globin RNA/mouse α -globin RNA $\pm$ S.E.	Adult Reticulocytes	Mean human β -globin RNA/mouse α -globin RNA $\pm$ S.E.	Copy no.	γ -globin RNA/transgene copy no. in adults	β -globin RNA/transgene copy no. in adults
		E10.5 Yolk sac	E13.5 Fetal liver	E10.5 Yolk sac	E13.5 Fetal liver		
μ LCR ^A $\gamma\psi\beta 8\beta$ with mtBP1	A	1.23 $\pm$ 0.04	0.12 $\pm$ 0.01	0.027 $\pm$ 0.01	0.65 $\pm$ 0.04	1.12 $\pm$ 0.12	1.12
	B	1.10 $\pm$ 0.02	0.08 $\pm$ 0.02	0.041 $\pm$ 0.01	0.40 $\pm$ 0.02	0.70 $\pm$ 0.15	1.4
	C	1.12 $\pm$ 0.02	0.22 $\pm$ 0.04	0.046 $\pm$ 0.01	0.80 $\pm$ 0.02	1.23 $\pm$ 0.13	0.6
	Mean $\pm$ S.E.	1.15 $\pm$ 0.04	0.14 $\pm$ 0.04	0.038 $\pm$ 0.01	0.62 $\pm$ 0.12	1.02 $\pm$ 0.16	1.04 $\pm$ 0.23
μ LCR ^A $\gamma\psi\beta 8\beta$ with wtBP1	A	N.D.	N.D.	N.D.	N.D.	0.90 $\pm$ 0.10	
	B	1.27 $\pm$ 0.02	0.13 $\pm$ 0.01	0.0013 $\pm$ 0.00	0.23 $\pm$ 0.03	0.73 $\pm$ 0.05	
	C	1.33 $\pm$ 0.04	0.11 $\pm$ 0.01	0.0020 $\pm$ 0.00	0.19 $\pm$ 0.01	0.80 $\pm$ 0.02	0.80
	Mean $\pm$ S.E.	1.29 $\pm$ 0.02	0.12 $\pm$ 0.01	0.0017 $\pm$ 0.00	0.21 $\pm$ 0.02	0.81 $\pm$ 0.05	