Developmentally Specific Role of the CCAAT Box in Regulation of Human γ-Globin Gene Expression*

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Abstract

The CCAAT box is a widespread motif in eukaryotic promoters. In this study we demonstrate that the effects of the CCAAT box on γ-globin gene activation are developmentally distinct. Although this promoter element is essential for high level γ gene expression in adult erythropoiesis, it plays little role in embryonic erythroid cells. The CCAAT mutation in the human γ-globin gene promoter impairs recruitment of TATA-binding protein (TBP), TFIIB, and RNA polymerase II in adult splenic erythroblasts but not in embryonic erythroid cells. We also show that the efficiency of γ gene transcription is correlated with recruitment of TBP on the TATA box but that the level of TBP recruitment is not nuclear factor Y (NF-Y)-dependent. Our data also suggest that it is unlikely that transcriptional stimulation by the CCAAT box is exerted through direct protein-protein interaction between NF-Y and TBP.

The CCAAT box is one of the most common promoter elements found in approximately one-third of eukaryotic housekeeping and lineage-specific genes (1). Other common cis elements in the minimal promoter are the TATA and CACCC boxes. The TATA box and the initiator compose the core promoter where the basal transcription apparatus docks. The CCAAT and CACCC boxes compose the proximal promoter that enhances RNA polymerase II (Pol II) transcription. Because of their widespread distribution and frequent co-existence in eukaryotic promoters, it has been speculated that the co-existence of CACCC and CCAAT boxes facilitates modulation of gene activity (2).

There are several examples of CCAAT mutations that cause a decrease in basal or lineage-specific promoter activities. Thus, disruption of the box C (NF-Y binding site) in the albumin gene promoter impairs expression in the liver (3). The transcription of farnesyl diphosphate synthase is up-regulated by sterol stimulation in the cell; point mutation in the CCAAT box of the farnesyl diphosphate synthase promoter disrupts sterol-dependent transcription in vivo (4). Transcription of the gene encoding E2F1, whose promoter contains three CCAAT motifs, is activated at the G1/S boundary; mutation of the proximal CCAAT motif has little effect on basal transcription but reduces transcription severalfold in S phase (5).

Each globin gene contains one or two CCAAT boxes (6). The CCAAT box is one of three prominent boxes found by saturation mutagenesis in the murine β-globin promoter (7). The CCAAT box of the human β-globin promoter binds CP-1 and NF-E1, and this binding is

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1The abbreviations used are: Pol II, RNA polymerase II; NF, nuclear factor; TBP, TATA-binding protein; ChIP, chromatin immunoprecipitation; CBF, CCAAT-binding factor; CREB, cAMP-response element-binding protein.
essential for γ gene expression induced by Me₂SO in MEL cells (8). Disruption of the CCAAT box of the human ε-globin gene resulted in a 3–4-fold decrease of ε expression at the embryonic stage of development in transgenic mice (9). The CCAAT box in the human γ-globin promoter has been investigated intensively (10–17) (for review, see Ref. 18) due in part to the fact that several mutations in the CCAAT box region produce the phenotype of hereditary persistence of fetal hemoglobin. The CCAAT box of the human γ-globin promoter has been identified as a positive cis element by various in vitro transcription and transient or stable transfection assays (11,14,19–22). Because hereditary persistence of fetal hemoglobin mutations in the CCAAT region activates the silenced γ gene in the adult, it is speculated that the CCAAT-binding proteins may function as repressors (15,17,21). Using gel shift assays several proteins have been characterized as γCCAAT region-binding factors: NF-Y (CP-1, CBF), CCAAT/enhancer-binding protein, CCAAT transcription factor/NF1, CCAAT displacement protein, NF-E3, and GATA1. Of these, only NF-Y has been confirmed as an in vivo γCCAAT-binding factor (20). CCAAT displacement protein, GATA1, and NF-E3 have been implicated in γ gene silencing. However, Ronchi et al. (17) have reported that disruption of GATA1 or NF-E3 binding in the context of a 70-kb β-globin locus cosmid could not reactivate γ gene expression in adult transgenic mice, suggesting that these two factors are unlikely to function as γ gene repressors; they also showed that the CCAAT box had little effect on embryonic/fetal globin gene expression (17).

To delineate the in vivo role of the CCAAT box of the γ gene promoter, we produced a transgenic mouse model in which the human γ gene is expressed in high levels during embryonic/fetal development as well as in adult life. The effects of mutations in the γCCAAT box could therefore be monitored at the various developmental stages. Our results showed that the effects of the CCAAT box on γ-globin gene activation are developmentally distinct. Although this promoter element is essential for high level γ gene expression in adult erythropoiesis, it plays little role in embryonic erythroid cells. We also showed that the efficiency of γ gene transcription is correlated with recruitment of TBP on the TATA box but that the level of TBP recruitment is not dependent on recruitment of NF-Y on the CCAAT box. Furthermore, our data suggest that direct protein-protein contacts between NF-Y and TBP in erythroid cells are unlikely.

**MATERIALS AND METHODS**

**Constructs, Transgenic Mice, RNA, and DNA Analysis**

Construct μLCR(−382)γ, which contained a μLCR-linked human γ gene from −382 (StuI) to +1950 (HindIII), has been described previously (23). Plasmid μLCR(−382)γ(mut CCAAT) was constructed by replacing the duplicated CCAAT box sequence “ACCAAT” with the sequence “AG-ATCT” (a BglII site) through PCR-based mutagenesis (20). Production of transgenic mice, RNase protection assay, and copy number measurement were described previously (24). Briefly, the construct μLCR(−382)γ(mut CCAAT) was microinjected into mouse eggs, and transgenic founders were identified by slot blot hybridization. Copy number of the transgene in the offspring was determined by Southern hybridization using human genomic DNA as standard and a mouse α-globin probe as loading control. Total RNA was prepared from tissues containing the primitive erythrocytes (day-10 and -12 blood and yolk sac) and tissues containing the definitive erythrocytes (day-12 and -16 fetal liver, day-16 blood, and adult blood). γ-Globin mRNA was detected by RNase protection assay and quantified by a PhosphorImager. To minimize experimental error, samples from individual animals were quantified independently, and multiple measurements were performed with both the RNase protection and Southern hybridization assays.
Chromatin Immunoprecipitation (ChIP) Assay

ChIP assays were performed as described previously with minor modifications (25,26). The tissues from the transgenic animals were passed through a 21-gauge needle in 5–10 ml of fresh RPMI 1640 medium and then cross-linked immediately. Protein-DNA cross-linking was performed by incubating cell suspensions with formaldehyde at a final concentration of 1.0% for 10 min at 37 °C with gentle agitation. One spleen, or brain, and six yolk sacs were used/condition. Glycine was added to 0.125 M to quench the reaction. Cells were then collected by centrifugation at 900 × g for 5 min and washed twice with cold phosphate-buffered saline. Nuclei were isolated by incubation in cell lysis buffer (10 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.2% Nonidet P-40) containing 1x protease inhibitor mixture (Roche Applied Science) for 10 min on ice followed by centrifugation at 600 × g for 5 min. Nuclei were lysed in nuclei lysis buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.5% SDS) with 1x protease inhibitor mixture for 10 min on ice. The lysate was sonicated with 15–20 pulses of 30 s each at 30–40% of maximum power with Fisher Sonic Dismembrator 550 equipped with a microtip to reduce the chromatin fragments to an average size of around 500 bp. The chromatin-DNA complex samples were diluted 5-fold with immunoprecipitation dilution buffer (14 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 0.4 mM EDTA, 0.01% SDS, 1.2% Triton X-100) and then aliquoted for immunoprecipitation. The soluble chromatin-DNA complex was precleared with normal IgG followed by protein G-Sepharose. The precleared chromatin was incubated with 1–5 μg of specific antibody, normal IgG in a final volume of 1 ml for 4–8 h at 4 °C. Immune complexes were collected by incubation with 50 μl of protein G-Sepharose for 2 h at 4 °C. Protein G-Sepharose pellets were washed twice with 500-μl aliquots of immunoprecipitation wash buffer 1 (20 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 0.4 mM EDTA, 0.1% SDS, 1% Triton X-100) and once with immunoprecipitation wash buffer 2 (10 mM Tris-HCl (pH 8.0), 0.25 M LiCl, 1 mM EDTA, 1% Nonidet P-40, 1% sodium deoxycholate). Chromatin DNA was then recovered and purified for quantitative PCR analysis by using the DNeasy™ tissue kit (Qiagen, Valencia, CA) according to the manufacturer’s directions. The DNA concentration was determined with a TK100 fluorometer (Hoefer Scientific Instruments, San Francisco, CA). About 1–20 ng of immunoprecipitated DNA was used as template in each PCR reaction in a total volume of 10 μl (26). Normal rabbit IgG (sc-2027), normal goat IgG (sc-2028), anti-NF-Y (CBF-B (C-18), sc-7712), anti-TBP (TFIID (TBP) (SI-1), sc-273), anti-TFIIB (TFIIB (SI-1), sc-274), and anti-Pol II (Pol II (N-20), sc-899) antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Quantitative PCR

Real time quantitative PCR was performed on the purified chromatin samples using the Roche LightCycler system (Roche Applied Science). PCR reactions were performed using the Fast-Start SYBR-Green Master kit (Roche Applied Science) according to the manufacturer’s instructions. The primer pairs used were designed according to the specific sequence of target DNA based on human and mouse β-globin sequences (GenBank™/EBI accession numbers U01317 and X14061) by using a program for primer design on-line (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The following primer pairs were designed and used in this work: human γ promoter region, 5′-CCCTTCCCCACACTATCTCA-3′ (upper strand) and 5′-GTGTGGAACTGCTGAAGG-3′ (lower strand); human γ CCAAT box, 5′-CCCTTCCCCACACTATCTCA-3′ (upper strand) and 5′-CCTGGCCTCACTGGATACTC-3′ (lower strand); human γ TATA box, 5′-GGCTGGCTAGGGATGAAGA-3′ (upper strand) and 5′-GGCGTCTGGACTAGGAGCTT-3′ (lower strand); mouse γ gene promoter region, 5′-GTT-GAAGGAGGAGCCAAAAA-3′ (upper strand) and 5′-GGCGTCTGGACTAGGAGCTT-3′ (lower strand); mouse γ gene promoter region, 5′-GTCATCACCGAAGCCTGATT-3′ (upper strand) and 5′-TTGCTGT-TTCTGGGGTTGTG-3′ (lower strand).
RESULTS

The CCAAT Box Is Essential for γ-Globin Gene Expression in Adult Erythropoiesis but Not in Embryonic Erythropoiesis

To assess the effects of CCAAT box on γ gene expression, a CCAAT box mutation was produced in the context of a μLCR(−382)Aγ construct (20), the wild form of which is expressed in all stages of development of transgenic mice (23). Five transgenic mouse lines carrying intact copies of the mutant CCAAT transgene were used for functional analysis. All RNase protection analyses for γ gene expression were performed on F2 animals, and the mean values were calculated from measurements of individual samples from the five lines. To assess the effects of CCAAT box mutation in embryonic erythropoiesis, we measured γ gene expression in 10- and 12-days postconception blood and yolk sac. The day-10 blood and yolk sac represent an early stage of embryonic erythropoiesis. At day 12, the yolk sac still contains large numbers of nucleated embryonic red cells, and the fetal blood is composed predominantly of nucleated erythrocytes of yolk sac origin. Fig. 1 shows a representative result of RNase protection assay, and quantitative data from PhosphorImager measurements of the assay are presented in the tables. As shown in Table I, the levels of γ mRNA in day-10 blood and yolk sac and in day-12 blood samples were essentially the same as in the control transgenic mice carrying a promoter with the wild-type CCAAT box. Apparently, the CCAAT box mutation has little effect on γ gene expression at the embryonic stage, in agreement with previous observations by Ronchi et al. (17).

To assess the effect of the CCAAT mutation in definitive erythropoiesis, we measured γ gene expression in day-12 fetal liver, in day-16 blood and fetal liver, and in adult blood. The day-12 fetal liver is composed of definitive erythroblasts and a small proportion (less than 10%) of hematogenously contaminating embryonic erythroblasts. At day 16, the blood and fetal liver contain mostly definitive red cells and erythroblasts. The adult blood contains erythroid cells originating from bone marrow. As shown in Table II, the CCAAT mutation did not affect γ gene expression in the day-12 fetal liver, and it resulted in a moderate decrease in the day-16 blood and fetal liver. In contrast, γ gene expression in the adult blood of the CCAAT box-mutant mice was reduced by about 3.5-fold compared with the control mice, suggesting that an intact CCAAT box is required for γ-globin gene expression in adult erythropoiesis.

The CCAAT Box Is Necessary for NF-Y Recruitment to γ-Globin Gene Promoter in Vivo

Previous studies have shown that the NF-Y recruitment is associated with high level γ gene expression in MEL cells stably transfected with a μLCR Aγ construct (20). The studies summarized above show that introduction of mutations to the CCAAT box did not affect the γ gene in embryonic erythroid cells. These observations raise the following question: Does NF-Y play a role in γ gene expression in embryonic erythropoiesis? To address this question we compared, by ChIP assay, NF-Y recruitment in day-11 embryonic erythroid cells and in adult splenic erythroblasts of wild and CCAAT-mutant mice. The majority of splenic cells of phenylhydrazine-treated animals are adult erythroblasts (27). The DNA brought down by the anti-NF-Y antibody was quantitated by real time PCR with a set of primers spanning the CCAAT box regions of the human Aγ promoter. In this experiment, the murine adult f(maj)-globin gene promoter was used as an internal control. The disruption of the CCAAT box reduced the recruitment of NF-Y to 30% in the adult spleen of mutant compared with the control mice (Fig. 2B). Using the same method, we assessed the effect of the CCAAT box mutation on recruitment of NF-Y in day-11 blood and yolk sac. In this case NF-Y recruitment in the murine embryonic εγ-globin promoter was used as the internal control. As shown in Fig. 2A, NF-Y recruitment on the CCAAT box-mutated γ promoter was reduced to 50% of the wild-type control in embryonic cells. These results suggest that even when NF-Y recruitment is impaired by the CCAAT mutation, γ gene expression can reach a high level in embryonic erythrocytes.
The Effect of the CCAAT Box Mutation on the Recruitment of Basal Transcription Machinery Is Developmental Stage-specific

Previous studies suggest that the CCAAT-facilitated TBP recruitment might be responsible for the enhanced \( \gamma \) gene expression in stably transfected MEL cells (20). Here we show that in embryonic erythrocytes the CCAAT box mutation did not affect \( \gamma \) gene expression. Two explanations could reconcile these observations. Either TBP recruitment is dependent on the CCAAT box, but transcription efficiency is not directly associated with the level of TBP recruitment (28); or transcription efficiency is associated with TBP recruitment, but the TBP binding is not directly dependent on the CCAAT box. To examine whether recruitment of TBP is dependent on the CCAAT box, we measured the recruitment of TBP in the CCAAT box-mutant transgenic mice. The DNA brought down by the anti-TBP antibody was quantitated by real time PCR with a set of primers spanning the TATA box regions of the human \( \alpha_\gamma \) promoter. The disruption of the CCAAT box reduced the recruitment of TBP to 30% in the adult spleen compared with the control (Fig. 3B). In contrast, disruption of the \( \gamma \)CCAAT box had no effects on the recruitment of TBP in the embryonic erythrocytes (Fig. 3A). This observation suggested that in embryonic erythrocytes the TBP binding is not correlated with the recruitment of NF-Y. Similarly, the recruitment of TFIIIB and Pol II to the \( \gamma \) promoter was affected only in the adult (Fig. 3, D and F) but not in embryonic cells (Fig. 3, C and E). The results suggest that recruitment of TBP, TFIIIB, and Pol II to the core promoter and recruitment of NF-Y to the CCAAT box are not mutually interdependent. Therefore, it is unlikely that a direct interaction between NF-Y and the basal transcription apparatus occurs in embryonic erythroid cells. However, the data did not exclude the possibility that a direct NF-Y/TBP contact might occur in adult erythropoiesis, resulting in enhanced \( \gamma \) gene expression.

NF-Y Does Not Directly Contact with TBP in Vivo

To test whether TBP recruitment and NF-Y recruitment are directly interdependent in adult erythropoiesis, we designed a modified ChIP assay (Fig. 4A). There is a NgoMI restriction site between the CCAAT and TATA boxes of the \( \gamma \) gene promoter. These two regions can be detected independently by different sets of PCR primers after NgoMI cleavage. With this approach NF-Y and TBP recruitment can be assessed independently. If recruitment of NF-Y and recruitment of TBP are interdependent through direct protein-protein contact, this association will result in detection of the TATA box in the chromatin pulled down by NF-Y antibody after NgoMI digestion, and vice versa. We performed this experiment using adult splenic erythroblasts of the transgenic mice carrying the wild \( \mu \) LCR(−382)\( \alpha_\gamma \) construct. The \( \gamma \) gene is expressed in high levels in this tissue. As expected, the same amounts of the CCAAT and TATA fragments can be detected in the chromatin pulled down by the NF-Y antibody before NgoMI digestion (Fig. 4B). After NgoMI cleavage the CCAAT box could be quantitatively recovered, but the TATA box was recovered by only 20% of the control (Fig. 4B). In the reciprocal experiment the TATA fragment could be completely recovered in the TBP antibody pull-down chromatin, whereas only about 20% of the CCAAT fragment was recovered after NgoMI cleavage (Fig. 4C). The cleavage efficiency of NgoMI on the cross-linked chromatin was ~80%. To ensure that the results are NgoMI cleavage-specific, we repeated the experiment using another restriction enzyme, BamHI, which does not cleave between the two boxes. In this case, the TATA and CCAAT fragments were recovered in equal amounts either in the TBP pulled-down or NF-Y pulled-down chromatin (Fig. 4, D and E). These results suggest that there is no direct contact between NF-Y and TBP in adult erythroid cells.

DISCUSSION

In this study we demonstrate that the effects of the CCAAT box on \( \gamma \)-globin gene activation are developmental stage-specific. Although this promoter element is essential for high level
γ gene expression in adult erythropoiesis, it plays little role in embryonic erythroid cells. We also show that the efficiency of γ gene transcription is correlated with recruitment of TBP on the TATA box but that the level of TBP recruitment is not dependent on recruitment of NF-Y on the CCAAT box. Furthermore, our data suggest that it is unlikely that there exists direct protein-protein contact between NF-Y and TBP in erythroid cells.

The developmentally specific role of the CCAAT box could reconcile apparently contradictory observations; studies using transfection assays suggest that the CCAAT box is indispensable in gene activation (2), whereas an in vivo study (17) suggests that the box is not essential. Our results show that the role of the CCAAT box on γ gene activity is dependent on the stage of development; in adult erythroid cells the CCAAT box exerts a positive effect on γ gene expression, whereas it has little influence in embryonic erythroid cells. The majority of studies on CCAAT box function using transfection assays have been carried out in cell lines originated from primary definitive cells; the transcriptional milieu of such cells most likely represents the trans acting environment of adult cells. This may explain why studies performed in cell lines always show a positive role of the CCAAT box in gene activation, i.e. the function we observed in vivo in the erythroid cells of adult transgenic mice.

Only a few studies have addressed the mechanism whereby NF-Y enhances gene transcription. It has been reported that NF-Y is able to directly associate with TBP in solution (29). This association involves the HS2 helix of TBP and the short stretches adjacent to the putative histone fold motifs of NF-YB and NF-YC. In addition to TBP, interaction between NF-Y and various TBP-associated factors also was detected in a protein-protein association assay (30). Our results showed that although the CCAAT mutant reduced NF-Y recruitment, it did not affect TBP recruitment in embryonic erythroid cells, suggesting that TBP recruitment is not directly facilitated by the CCAAT box-bound NF-Y. The CCAAT mutant does simultaneously impair recruitment of NF-Y and TBP in adult erythroblasts. However, ChIP assays failed to detect direct contact between TBP and NF-Y. Based on these observations we conclude that it is unlikely that the CCAAT-enhanced γ gene activation is exerted through direct interaction between TBP and NF-Y.

We have reported that the CCAAT box mutation resulted in a reduction of chromatin accessibility of the γ gene promoter in stably transfected MEL cells (20), and a similar decrease also was observed in adult splenic erythroblasts. Binding of NF-Y on the CCAAT box is able to distort the double helix by angles of 62–82° (31). In addition to this direct effect, NF-Y is able to tether to a promoter histone acetyltransferase p300, p300/CREB-binding protein-associated factor, or GCN5 (32,33). Jin and Scotto (34) reported that treatment with trichostatin A resulted in a 20-fold increase of expression of the MDR1 gene and that the CCAAT box of the MDR1 gene promoter mediated this effect. Similar results were observed in the hsp70 promoter in Xenopus oocyte nuclei (35). These observations suggest that recruitment of NF-Y to the CCAAT box may establish a preset promoter architecture that can facilitate RNA polymerase II transcription. This hypothesis is in agreement with our observations, which suggest that CCAAT-mediated transcription enhancement may not be mediated by direct contact between NF-Y and the basal transcription apparatus.

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2X. Fang, G. Stamatoyannopoulos, and Q. Li, unpublished data.
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Fig. 1. Human γ-globin RNA quantitation by RNase protection assay

A representative result is presented in this figure. Erythroid tissues were collected from four individual transgenic embryos (labeled as 1–4) and from four 3-week-old individual mice (labeled as 5–8) carrying the construct μLCRγ (mut CCAAT). The day-12 blood (Bl) and yolk sac (YS) samples are composed of embryonic erythrocytes. The day-12 fetal liver (FL) and adult (Ad) blood is composed of definitive erythrocytes. Total RNA was isolated and hybridized with an RNA probe mix containing the antisense sequences of human γ-globin exon 2 and mouse α- and ζ-globin exon 1. Protected fragments and sizes are as follows: human γ-globin (Hu γ), 170 bp; mouse ζ-globin (Mu ζ), 151 bp; and mouse α-globin (Mu α), 128 bp. Intensities of the protected bands were measured by a PhosphorImager. Expression levels of the human γ gene were normalized by endogenous mouse α-like globin mRNAs and corrected by copy numbers (see “Materials and Methods”).
Fig. 2. Recruitment of NF-Y in transgenic mice carrying the CCAAT box-mutated γ-globin gene
NF-Y recruitment on the γ promoter was measured by real time PCR-based ChIP assay. The
day-11 blood and yolk sac (marked as “Embryo” in A) and the adult spleen (marked as “Adult” in B) were harvested from transgenic mice carrying the wild-type γ promoter (WT) or the mutant γ construct (CCAAT mut). Formaldehyde was added to cell suspension, and cross-linked chromatin was solubilized by sonication. Soluble chromatin was subjected to immunoprecipitation with the antibody against NF-Y. The γCCAAT box in the recovered chromatin was quantitated by real time PCR. Data presented in this figure were generated from three independent immunoprecipitations and duplicate DNA quantitation. The endogenous εy promoter or βmaj promoter served as internal control in the embryonic or adult samples. Nonspecific IP control was performed in the chromatin of the wild-type mice and expressed as a percentage of immunoprecipitation from specific antibody. Non-expressing tissue control was performed in the brain of the wild-type mice immunoprecipitated by anti-NF-Y and expressed as a percentage of immunoprecipitation in erythroid tissues. The effect of the CCAAT box mutation on NF-Y recruitment was calculated by dividing NF-Y recruitment on
the mutated promoter over that on the βmaj promoter in the CCAAT-mutant mice by the same ratio in the control mice. All data were corrected by the copy numbers of the transgenes.
Fig. 3. Recruitment of components of the basal transcription apparatus in transgenic mice carrying the CCAAT box-mutated γ-globin gene

TBP (A and B), TFIIB (C and D), and Pol II (E and F) recruitment on the γ promoter was measured by ChIP assay as described in the legend of Fig. 2. The embryonic and adult stages are indicated at the top of the left and right column panels, respectively. The antibodies used in each analysis are indicated inside the panels. WT, wild-type γ promoter; CCAAT mut, mutant γ construct.
Fig. 4. Analysis of NF-Y/TBP interaction by a modified ChIP assay

A, diagram showing the ChIP assay designed for detection of protein-protein interaction between NF-Y and TBP. If physical contact between NF-Y and TBP occurs in the cells, the two proteins would be cross-linked by formaldehyde. After NgoMI cleavage, both the TATA and CCAAT fragments should be detected in the chromatin immuno-precipitated either by the anti-TBP or by anti-NF-Y antibodies. If the contact is absent, the TATA fragment should be present only in chromatin pulled down by the anti-TBP antibody and absent in chromatin pulled down by the anti-NF-Y antibody and vice versa. B, measurements of the CCAAT and TATA fragments in the chromatin immuno-precipitated by anti-NF-Y antibody before (filled bars) and after (open bars) NgoMI digestion. C, measurements of the CCAAT and TATA fragments in the chromatin immuno-precipitated by anti-TBP antibody before (filled bars) and after (open bars) NgoMI digestion. D, measurements of the CCAAT and TATA fragments in the chromatin immuno-precipitated by anti-NF-Y antibody before (filled bars) and after (open bars) BamHI digestion. E, measurements of the CCAAT and TATA fragments in the chromatin immuno-precipitated by anti-TBP antibody before (filled bars) and after (open bars) BamHI digestion.
in the chromatin immunoprecipitated by anti-TBP antibody before (filled bars) and after (open bars) NgoMI digestion. D, measurements of the CCAAT and TATA fragments in the chromatin immuno-precipitated by anti-NF-Y antibody before (filled bars) and after (open bars) BamHI digestion. E, measurements of the CCAAT and TATA fragments in the chromatin immunoprecipitated by anti-TBP antibody before (filled bars) and after (open bars) BamHI digestion.
Table I

Human γ-globin gene expression in embryonic erythropoesis of transgenic mice carrying the construct μLCR(−382)γ(mut CCAAT)

| Line  | Copy number | Day 10   | Day 12   |
|-------|-------------|----------|----------|
|       |             | Blood    | Yolk sac | Blood    | Yolk sac |
| μLCR(−382)γ(mut CCAAT) |             |          |          |          |          |
| A     | 4           | 3.6 ± 1.2| 6.8 ± 2.0| 12.5 ± 3.4| 16.6 ± 1.4|
| B     | 3           | 4.7 ± 1.6| 8.0 ± 1.5| 6.6 ± 1.9 | 10.1 ± 1.2|
| C     | 14          | 5.5 ± 1.5| 9.5 ± 1.7| 6.1 ± 0.4 | 8.0 ± 0.5 |
| D     | 2           |          | 6.8 ± 2.3| 8.0 ± 3.3 |
| E     | 12          | 5.6 ± 0.5| 11.3 ± 2.1| 7.9      | 10.0     |
| Mean  |             | 4.9 ± 0.9| 8.9 ± 1.9| 8.0 ± 2.6 | 10.5 ± 3.5|
| μLCR(−382)γα |             |          |          |          |          |
| Mean  |             | 5.7 ± 6.3| 5.2 ± 3.3| 10.4 ± 9.5| 21.0 ± 7.4|

* Cited from Ref. 23.
Table II

Human γ-globin gene expression in definitive erythropoiesis of transgenic mice carrying the construct μLCR(−382)γ(mut CCAAT)

| Line   | Copy number | Day 12 | Day 16 | Adult blood |
|--------|-------------|--------|--------|-------------|
|        |             | Liver  | Blood  | Liver       |
| μLCR(−382)γ(mut CCAAT) |             |        |        |             |
| A      | 4           | 12.0 ± 2.5 | 10.8 ± 5.1 | 8.8 ± 3.9 | 7.6 ± 0.6 |
| B      | 3           | 15.3 ± 4.3 | 7.4 ± 2.9  | 10.2 ± 3.5 | 3.6 ± 0.5 |
| C      | 14          | 9.2 ± 1.6  | 11.1 ± 3.8 | 11.6 ± 1.7 | 3.6 ± 1.0 |
| D      | 2           | 12.1 ± 3.7 | 10.6     | 8.0        | 3.6 ± 0.2 |
| E      | 12          | 9.6      | 8.1 ± 2.0 | 10.2 ± 1.6 | 5.1 ± 0.8 |
| Mean   |             | 11.6 ± 2.4 | 9.6 ± 1.7 | 9.8 ± 1.4 | 4.7 ± 1.7 |

| μLCR(−382)γα |             |        |        |             |
| Mean         |             | 11.8 ± 2.8 | 13.4 ± 4.7 | 15.7 ± 4.8 | 16.5 ± 4.5 |

*a* Cited from Ref. 23.