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Expression of GATA-1 in a Non-Hematopoietic Cell Line Induces β -Globin Locus Control Region Chromatin Structure Remodeling and an Erythroid Pattern of Gene Expression

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Abstract

GATA-1 is a hematopoietic transcription factor expressed in erythroid, megakaryocytic, mast cell and eosinophil lineages. It is required for normal erythroid differentiation, the expression of erythroid-specific genes and for the establishment of an active chromatin structure throughout the β -globin gene locus. GATA-1 is also necessary for the formation and function of the locus control region DNase I hypersensitive site (HS) core elements. To determine whether GATA-1 was sufficient to direct locus control region (LCR) formation and an erythroid pattern of gene expression, we expressed GATA-1 in the non-hematopoietic Hela cell line which does not express other hematopoietic transcription factors but does express GATA-2, GATA-3 and GATA-6. We found that GATA-1 protein production resulted in the formation of LCR DNase I HSs 1-4 in their normal locations and that histones became hyperacetylated within these regulatory elements. Transcription of several erythroid-specific genes was also activated in Hela cells expressing GATA-1, including those coding for α -globin, β -globin, the erythropoietin receptor, EKLF and p45 NF-E2. Despite increased expression of these genes at the mRNA level, their protein products were not detected. These results imply that GATA-1 is sufficient to direct chromatin structure reorganization within the β -globin LCR and an erythroid pattern of gene expression in the absence of other hematopoietic transcription factors.

INTRODUCTION

GATA-1 is an hematopoietic transcription factor that is expressed in erythroid, megakaryocytic, eosinophil and mast cell precursors ¹. GATA-1 contains two conserved zinc-finger motifs which mediate binding to WGATAR consensus sequences and protein-protein interactions with several other transcription factors including Sp1, CBP/p300, FOG-1 and the erythroid-specific factors EKLF and GATA-1, itself ². GATA-1's role in terminal erythroid differentiation includes suppression of GATA-2 expression, auto-regulation of its own expression, and up-regulation of erythroid-specific target genes including those for erythroid krüppel-like factor (EKLF), NF-E2, the α - and β -globins and the erythropoietin receptor. Knock-out of the GATA-1 gene in mice results in severely impaired erythrocyte production through a block to differentiation at the proerythroblast stage and a lethal anemia prior to birth

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3; 4. Natural mutations in the human GATA-1 gene have been described in several relatively rare disease syndromes including congenital dyserythropoietic anemia⁵, macrothrombocytopenia^{6; 7; 8} and megakaryocytic leukemia in children with Down's syndrome⁶. GATA binding sites are found in the regulatory elements of most, if not all, erythroid genes, including promoters, silencers, enhancers and, a primary focus of this report, the β -globin locus control region⁹.

While the β -globin LCR spans more than 20 kb, its five functional elements are contained within several hundred base pair domains of unique chromatin structure which were initially characterized as DNase I hypersensitive sites (HSs). LCR HSs 2, 3 and 4 are able to confer position-independent expression on linked genes in transgenic mice^{10; 11; 12} and have been used to achieve consistent, high-level expression in the current generation of lentiviral globin gene transfer vectors that are now moving toward clinical trials^{13; 14}. HS5 is not erythroid-specific and has properties of a chromatin insulator element^{15; 16; 17}. Loss or mutation of the LCR HSs has been studied in a variety of systems including knock-out mice, human β -globin YAC transgenic mice and naturally occurring human mutations¹⁸. While the results from these models differ in some respects, a consistent finding is that the LCR HSs are required for normal, high-level expression of the β -globin locus genes. LCRs have been found in several other gene loci and their mechanism of action is the subject of active investigation¹⁹.

We previously determined that tandem-inverted GATA binding elements located within the cores of β -globin LCR HSs 1-4 are required for HS formation^{20; 21}. These HSs are erythroid-specific, approximately 150 bp in size and are characterized by unique sensitivity to DNase I, being 10-20 fold more sensitive than the promoter HSs of the β -globin locus^{22; 23}. Formation of the HSs is also dependent on the presence of an NF-E2 binding element located approximately 50 bp upstream of the GATA binding elements and, at least in the case of LCR HS4, two krüppel-like factor binding sites^{20; 24}. More recently, GATA-1 has been shown to be required for hyperacetylation of histones associated with the β -globin LCR HSs²⁵. Because GATA-1 is the only GATA factor expressed in mature erythroid cells, is able to interact with many other proteins and is required for the formation and function of the LCR HSs, we hypothesized that GATA-1 was the critical factor involved in the formation of the functional elements of the β -globin LCR. The fact that GATA-1 is also required for the expression of most, if not all erythroid-specific genes, lead us to hypothesize that GATA-1 could serve as a "master regulator" of erythroid gene expression and might, like the well-known example of MyoD, activate tissue specific gene expression and confer a tissue-specific phenotype when expressed in cells of other lineages²⁶.

To test these hypotheses, we have expressed GATA-1 in the non-hematopoietic Hela cell line. While several previous studies have examined the effect of ectopic GATA-1 expression on cellular phenotype and gene expression patterns, these studies have been performed in hematopoietic cell lines that contain other hematopoietic transcription factors and intact signaling pathways. Kulesa, et al²⁷ expressed GATA-1 in transformed avian myeloblastic cell lines and under various conditions found phenotypic conversion to erythroid, eosinophilic or megakaryocytic cell types. Joneja, et al²⁸ expressed GATA-1 in the murine myeloid FDCP2 cell line and observed no change in phenotype, but did see alterations in intracellular signaling and induction of EKLF and β -major globin expression. Yamaguchi, et al,²⁹ found that forced GATA-1 expression in the M1 murine myeloid leukemia cell line resulted in the formation of mixtures of megakaryocytic and erythroid cells within individual clones and activation of erythroid and megakaryocytic genes. Hela cells, which were originally derived from a cervical adenocarcinoma³⁰, are not hematopoietic in origin, should not express other erythroid or hematopoietic transcription factors and therefore should provide a more stringent test of whether GATA-1 is independently capable of directing LCR HS formation in the absence of

other hematopoietic transcription factors and whether forced GATA-1 expression will induce other erythroid genes.

METHODS

Cell Culture

K562 and MEL cells were grown in IMEM and Hela S3 cells were grown in DMEM, both supplemented with 10% fetal calf serum. To analyze cells for the presence of hemoglobin, 0.2% w/v benzidine hydrochloride was prepared in 3% v/v glacial acetic acid. Immediately prior to use 1% v/v of 30% hydrogen peroxide was added. Cells in growth media were then diluted 1:1 in the stain and incubated for 30 minutes at room temperature before viewing³¹. For stable transfection of the GATA-1 gene into Hela cells, GATA-1 cDNA (gift of Dr. Stuart Orkin) was sub-cloned into the pRC-CMV plasmid (Invitrogen). Linearized vector was then electroporated into Hela cells using the Gene Pulser system (Biorad) at settings of 950 μ F and 220 mV. Individual clones containing the vector were selected and expanded in 800 μ g/ml G418 over 3-4 weeks.

Protein and mRNA analysis

Western blotting was performed using standard techniques with the following antibodies from Santa Cruz Biotechnology: GATA-1, sc-265; GATA-2, sc-267; GATA-3, sc-268; GATA-6, sc-9055; NF-E2, sc-291 and EKLF, sc-27193. For reverse transcriptase PCR, RNA was isolated using RNeasy (Qiagen). Integrity of the RNA sample was confirmed by agarose gel electrophoresis. For each PCR reaction, 1 μ g of total RNA was reverse transcribed using Murine Leukemia Virus reverse transcriptase provided with the Gene AMP RNA PCR kit (Applied Biosystems). cDNA samples were amplified with gene specific primers in the presence of approximately 0.5 μ Ci 32 P- α -dCTP added at the beginning of the reaction. PCR products were visualized following polyacrylamide gel electrophoresis. The following PCR primers spanned intron-exon boundaries: β -actin 5'-GGGACCTGACCGACTACCTC-3' and 5'-GGGCGATGATCTTGATCTTC-3'; β -globin 5'-GGTGGTCTACCCTTGGACCC-3' and 5'-GATACTTGTGGGCCAGGGCA-3'; α -globin 5'-TGGGGTAAGGTTCGGCGCGCA-3' and 5'-TGCACCGCAGGGGTGAAGTC-3'; γ -globin 5'-GGAGGAGAAACCCTGGGAAG-3' and 5'-CCCAGGAGCTTGAAGTTCTC-3'; ϵ -globin 5'-TGGAGGTGAAGCCTTGGG-3' and 5'-AGTCAGCACCTTCTTGCC-3'; erythropoietin receptor 5'-GCACCGAGTGTGTGCTGACGAA-3' and 5'-GGTCAGCAGCACCAGCATGAC-3'; LMO2 5'-GGATCCTGCCGGAGAGACTATCTC-3' and 5'-GAATTCAGTGAACACCTCCGCAAA-3'; EKLF 5'-CACTAGGAGAGTCCAAAGTGCC-3' and 5'-TCAGTACCAAGGGCACTTCC-3'; NF-E2 5'-ACAGTACCAGTGCCCCGTGTCCTC-3' and 5'-AGACCAGCTCAATCTGTAGCCTCC-3'; FOG1 5'-TCGCCAGGACTGGACAGAAAG-3' and 5'-CTGTGCACCAGCTCGGACATC-3'; GATA-1 5'-TGAATCCTCTGCATCAACAAGC-3' and 5'-CAGTTGAGGCAGGGTAGAGTGC; SCL/TAL-1 5'-AATCGAGTGAAGAGGAGACC-3' and 5'-AATCGAGTGAAGAGGAGACC-3'.

Chromatin Structure Determination

Southern blot based DNase I HS assays were performed as previously described³² using DNase I (Worthington Biochemical) at concentrations from 0 to 10.0 mg/ml. Quantitative PCR based DNase I sensitivity assays were performed using the method of McArthur, et. al.³³ and DNase I concentrations from 0 to 20 μ g/ml. We also sonicated all DNA samples prior to Sybr Green Q-PCR to assure equivalent template length. AcH3 ChIP assays were performed using the Upstate protocol and anti-acetylated histone H3 antibody #06-599 (Upstate). GATA-1 ChIP assays were performed using the procedure of Nelson, et. al.³⁴ and Santa Cruz anti-GATA-1

antibody sc-265. Average fragment length was approximately 400bp. PCR primers for detection of GATA-1 binding and histone H3 acetylation at the β -globin LCR HS3 core were 5'-ATAGACCATGAGTAGAGGGCAGAC-3' and 5'-TGATCCTGAAAACATAGGAGTCAA-3'. Those for the LCR HS4 core were 5'-AAAAAAGAATTCCTTGCCCTGCCTCTCT-3' and 5'-AAAAAAGGATCCCTCGGCCCTGGCTCTG-3'. The HS4 primers were originally used to amplify and sub-clone the HS4 core element. The underlined portions are complimentary to the human HS4 region. Immunoprecipitation reactions were performed in triplicate using IgG as a non-specific control. The relative amount of target sequence DNA from each precipitation was determined in duplicate or triplicate by quantitative real-time PCR by comparison to a standard curve on input DNA using Sybr Green.

RESULTS

To test the effect of ectopically expressed GATA-1 on the chromatin structure of the human β -globin gene locus, the murine GATA-1 gene was sub-cloned into the pRC-CMV plasmid and transfected into HeLa S3 cells. The murine form of GATA-1 was chosen for these experiments, as it would allow us to follow the expression of both the ectopic mouse gene and the endogenous human GATA-1 gene. Clones with stably integrated plasmids were selected on the basis of neomycin resistance. Individual clones were then analyzed for mGATA-1 mRNA and protein expression. As seen in Figure 1, three representative clones express GATA-1 mRNA (Figure 1A) and protein (Figure 1B) at levels equivalent to those seen in MEL (murine) and K562 (human) erythroid cell lines. No expression was seen in the untransduced wild-type HeLa S3 cell line (HWT).

GATA-1 expression induces β -globin LCR HS formation and histone hyperacetylation

We initially analyzed the GATA-1 expressing cells for changes in morphology, growth characteristics and hemoglobin expression as judged by benzidine staining. We found no change in morphology or doubling time and neither the parental HeLa cell line or the GATA-1 expressing clones expressed detectable hemoglobin by benzidine staining (data not shown). We next asked whether ectopically expressed GATA-1 was capable of remodeling the chromatin structure of the human β -globin gene locus. To determine this, we first performed DNase I hypersensitive site assays to evaluate LCR HS formation in the GATA-1 expressing HeLa cells. Results of these experiments are shown in Figure 2. Figure 2B shows the restriction fragments and probes used to map each HS. In Figure 2A, HS assays for LCR HSs 1-4 are shown. The positive control for these experiments is the human K562 cell line. The expected LCR HSs are seen in these cells as previously described^{22; 23}. The negative control for these experiments is the parental HeLa cell line. When GATA-1 is expressed in HeLa cells, each LCR HS is formed at its expected location and with the a level of DNase sensitivity equivalent to that is seen in the K562 control cells. The only exception to the correct HS location, is that HS1 may form in a position slightly closer to HS2 in the GATA-1 HeLa cells. HS3, which is normally composed of three adjacent bands²⁰, shows these same features in the GATA-1 expressing HeLa cells. It can also be seen that HS2 is present WT HeLa cells. While this site has been previously reported to form in HeLa cells²³, it is present only at higher concentrations of DNase I. Note that in HeLa cells expressing GATA-1, HS2 is formed at lower concentrations of DNase I, similar to those seen in the K562 erythroid cells. In Figure 2C, we show a quantitative assay of DNase I sensitivity for HS3 using the method of McArthur, et al³³. While the region of H3 shows significant DNase I digestion in HeLa (HWT) cells only at the highest concentrations tested, both the region is much more sensitive in K562 and GATA-1 expressing HeLa cells (HG1). We have previously shown that the LCR DNase HSs are coincident with domains of positioned nucleosomes and that the center of the HS regions is associated with approximately 150 bp regions in which nucleosomes have been displaced or disrupted²⁰;

²¹. Our results indicate that ectopically expressed GATA-1 is able to independently mediate the formation of these sites in HeLa cells.

The murine LCR HS core regions were recently shown to contain hyperacetylated H3 and H4 histones ^{35; 36}. To determine whether GATA-1 is able to independently direct hyperacetylation of nucleosomes near the LCR HSs, we performed chromatin immunoprecipitation (ChIP) assays for acetylated histone H3 in the regions of LCR HS3 and HS4. The results of these experiments are shown in Figure 3. Precipitation reactions were performed and analyzed using quantitative PCR as described by Im, et al. ³⁷. The amount of precipitated DNA is compared to the non-specific IgG control for each region. In this experiment the same cell lines were used as positive and negative controls. In the K562 positive control cell line, there is enrichment of DNA associated with both HS3 and HS4 following acetylated histone H3 immunoprecipitation. The wild-type HeLa cell lines showed no histone H3 acetylation compared to the IgG controls at either of the LCR HSs. In HeLa cells expressing GATA-1, histone H3 hyperacetylation is detected at the LCR HSs indicating that GATA-1 expression is able to mediate not only DNase I HS formation, but also appropriate histone hyperacetylation in the regions of the HSs.

Ectopic expression of GATA-1 induces the expression of erythroid-specific genes

Because forced expression of GATA-1 is able to direct the formation of the active structures of the β -globin LCR in a non-hematopoietic cell line, we next asked whether expression of β -globin locus genes was also being activated. For these experiments, we used reverse-transcriptase RT-PCR. Human bone marrow and K562 cells served as positive controls and untransduced HeLa cells were the negative controls. As shown in Figure 4A, ectopic GATA-1 production preferentially activates expression of the adult β -globin gene in HeLa cells and, to a lesser extent, expression of the fetal γ -globin genes. The embryonic ϵ -globin gene is not expressed in this setting. To determine whether the activation of these erythroid genes was specific to the β -globin locus, we next analyzed the expression of several other known GATA-1 target genes. As shown in Figure 4B, expression of the α -globin, erythropoietin receptor (EpoR) and Tal-1 genes is activated in GATA-1 expressing cells, indicating that the effects of GATA-1 are not limited to the β -globin locus. Lmo2, a transcription factor which interacts with GATA-1, but whose expression does not appear to be regulated by GATA-1 ³⁸, is not up-regulated in HeLa cells expressing GATA-1. Although the primary focus of our work was to determine the effects of GATA-1 on erythroid genes, we also found that expression of the megakaryocyte-specific thrombopoietin receptor gene (c-MPL), another GATA-1 target gene, was also activated in HeLa cells expressing GATA-1 (data not shown).

The α -globin gene appears to be the most strongly induced of the tested genes. However, when we performed Western blot analysis to determine whether α -globin protein was produced, none was detected (data not shown). As a more functional assay for erythroid-specific protein production, we grew GATA-1 expressing HeLa cells in cell-free medium with and without added erythropoietin (Epo) to determine whether we could detect a change in the growth kinetics or hemoglobin production, which would indicate signaling through the EpoR. Both assays showed no effect of erythropoietin (data not shown).

Finding that GATA-1 induced the expression of several erythroid genes in HeLa cells raised the possibility that GATA-1 may not be independently directing LCR HS formation in our transfected HeLa cells, but that GATA-1 might be activating expression of EKLF and/or p45 NFE2, two known GATA-1 target genes. Although it is not clear what the actual role of these factors is in LCR HS formation, binding sites for these factors are necessary for normal LCR HS formation and function ^{20; 21} and the factors are known to bind LCR HS core elements in gel mobility shift assays ²⁴. To determine whether these proteins might be playing a role in LCR HS formation in GATA-1 expressing HeLa cells, we first performed RT PCR to determine

whether these genes were up-regulated at the mRNA level. As shown in Figure 5A, transcripts for both genes are detected in HeLa cells expressing GATA-1, but not in wild-type HeLa control cells. However, neither EKLF nor p45 NF-E2 protein is detected by Western blot (Figure 5B). This makes it unlikely that either protein is present in sufficient quantity to be involved in the formation of the β -globin LCR HSs detected in the HeLa cells expressing GATA-1.

Finally, we wanted to ask whether the ability of ectopically expressed GATA-1 to establish an erythroid pattern of β -globin LCR chromatin structure and gene expression was due to unique properties of GATA-1 or whether any GATA family member could establish these tissue-specific characteristics. To assess this, we performed Western blots to determine whether other GATA proteins were present in the specific HeLa S3 cells used in our experiments. Results of these blots are shown in Figure 6. Here we see that GATA-2, GATA-3 and GATA-6 are all detected in HeLa cells (Figure 6A) and that, in the case of GATA-6, the level is equivalent to that seen in the colon cancer cell line COLO 320 (Figure 6B). These results imply that, despite the fact that all of the GATA factors recognize similar binding sequences, GATA-1 is uniquely able to establish erythroid gene expression patterns and LCR HS formation.

DISCUSSION

GATA-1 is involved in the expression of most, if not all, erythroid-specific genes. It is also necessary for normal erythroid differentiation and for the reorganization of the chromatin structure of the β -globin gene locus²⁵. Previous studies evaluating GATA-1 function have been performed in erythroid or other hematopoietic cell types. Over-expression of GATA-1 revealed the ability of the factor to reprogram gene expression and cell phenotype in a pre-set hematopoietic context. Other strategies which eliminate or suppress GATA-1 levels in cells where it is usually produced have been used to determine which cellular functions GATA-1 is required for, but not what functions the protein is able to mediate in the absence of its associated hematopoietic transcription factors. To address this question we expressed GATA-1 in the non-hematopoietic HeLa cell line. One result of this ectopic expression was the formation of the LCR HSs in their normal locations and with their characteristically high level of sensitivity to DNase I. We have previously shown that binding elements for NF-E2 and EKLF are necessary for normal LCR HS formation²⁰ and that the tandem, inverted WGATAR binding elements found at the cores of each LCR HS are insufficient to form a HS on their own²⁴. Taken together, these results suggest that GATA-1 interacts with other non-erythroid DNA binding factors which are able to occupy sites near the LCR HS core WGATAR elements in HeLa cells. For example, the factors Sp1 and Sp3 have been shown to bind *krüppel*-like factor binding sites within the LCR HS core regions²⁴. Similarly, AP-1 is able to bind NF-E2 sites within the cores³⁹. Our results suggest that while GATA-1 alone is insufficient to form the LCR HSs, it can cooperate with nonerythroid factors to accomplish HS formation.

The role of GATA-1 in establishing regions of histone hyperacetylation in erythroid cells has been studied by Letting, et al,⁴⁰ and Kiekhaefer, et al,⁴¹ by re-expressing GATA-1 in erythroid cells deficient for this factor. While both groups found that GATA-1 was necessary to establish the domains of histone H3 and H4 hyperacetylation that co-localize with the LCR HSs, these results still left open the question of which erythroid factor or factors which bind to the HS cores (i.e., GATA-1, EKLF, NF-E2 or others) were responsible for the tissue specificity of the hyperacetylation pattern. Our results suggest that GATA-1 is the determining factor, as it is able to direct the formation of the LCR domains of hyperacetylation in a non-erythroid transcriptional environment.

In addition to the factors which bind DNA near GATA-1 in the LCR cores and other regulatory elements, GATA-1 is known to form complexes *in vivo* with a variety of other proteins^{2; 9}. Distinct positive and negative regulatory complexes have been isolated from erythroid cells

by co-immunoprecipitation⁴². It therefore seems likely that when expressed in HeLa cells, GATA-1 is interacting with non-erythroid proteins to alter LCR chromatin structure and induce erythroid gene expression. One potential interacting factor is the histone acetyltransferase, CREB-binding factor (CBP). This widely-expressed transcriptional co-regulator is present in HeLa cells^{43; 44}. It has been shown to augment GATA-1 target gene expression⁴⁵ and to be required for LCR activation of β -globin locus genes⁴⁶ and for establishing the erythroid histone acetylation pattern of the β -globin locus⁴⁰. CBP has been co-immunoprecipitated with GATA-1⁴⁵ and is able to activate GATA-1 through acetylation in the region of the zinc finger domains^{47; 48}. This acetylation may also enhance GATA-1's DNA binding affinity⁴⁸. Because CBP is implicated in several GATA-1 mediated functions and is expressed in HeLa cells, it is likely to be involved in GATA-1's effects in HeLa cells.

Although we have observed an induction of erythroid gene expression at the mRNA level, we have not been able to detect significant production of erythroid proteins except for GATA-1 itself. One possible explanation for this observation is that the levels of mRNA we have detected from the activated erythroid genes is insufficient to produce significant protein production. Both NF-E2 and EKLF have been shown to activate erythroid gene expression and are lacking in HeLa cells. Perhaps expressing these and other erythroid transcription factors in combination with GATA-1 could produce an erythroid phenotype.

Finally, we have shown that HeLa cells normally express several GATA family member proteins including GATA-2, GATA-3 and GATA-6. The fact that erythroid-specific gene expression and chromatin structures are not seen in HeLa wild-type cells implies that GATA-1 has unique functional properties which allow it to act in a tissue-specific fashion. This finding is in agreement with results from Takahashi, et al,⁴⁹ who replaced GATA-1 by GATA-2 or GATA-3 in the murine GATA-1 locus. They found that while the mice did have sufficient erythropoiesis to survive, they exhibited anemia as adults. The authors concluded that there were significant biochemical differences between the different GATA family members.

In summary, our results suggest that GATA-1 has the ability to activate an erythroid pattern of gene expression and direct the formation of an active chromatin structure at the β -globin LCR, but that other factors are also required to achieve a full erythroid phenotype.

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Figure 1.

Forced expression of GATA-1 in HeLa cells. A) RT PCR analysis shows that mGATA-1 is expressed in three HeLa clones (HG1, HG2 and HG3) stably transfected with a mGATA-1 expression plasmid. Levels are comparable to those seen in the MEL control cell line. No GATA-1 mRNA is detected in the wild-type HeLa cells (HWT). B) Western analysis of mGATA-1 expression. GATA-1 protein is expressed in each transfected clone at levels similar to those seen in MEL and K562 control cell lines.

Figure 2.

Human β -globin LCR HS formation in HeLa cells expressing GATA-1. A. DNase I HS assays for β -globin LCR HSs 1-4. K562 and HeLa wild-type cells serve as positive and negative controls. Arrows indicate DNase I HSs. B. DNA and fragments used in DNase I HS assays. Arrows below the maps indicate the expected size of HS bands on the Southern blots. C. Quantitative PCR based DNase I sensitivity assay used to analyze the region of LCR HS3. B = Bam HI, Sa = Sac I, Sp = Sph I, H = Hind III.

Figure 3.

Histone H3 acetylation and GATA-1 binding at the β -globin LCR HSs in HeLa cells expressing GATA-1. A. Levels of H3 acetylation in the LCR HS3 region. B. Levels of H3 acetylation in the LCR HS4 region. C. GATA-1 binding to the regions of LCR HS4 and HS3. K562 and HeLa wild-type cells are positive and negative controls for these experiments. IgG is used as a non-specific control for immunoprecipitation reactions. HG1 = GATA-1 expressing HeLa cells.

Figure 4.

Expression analysis of erythroid GATA-1 target genes in Hela cells expressing GATA-1 using reverse transcriptase-PCR. A. Expression of the human β -globin locus genes. B. Expression of non- β -globin locus genes. RNA from unfractionated human bone marrow and K562 cells are positive controls. EpoR, erythropoietin receptor gene. HG1, Hela cells expressing GATA-1.

Figure 5.

EKLf and p45 NF-E2 expression in HeLa cells producing GATA-1. A. RT-PCR showing that message for both genes is detectable. B. Western blot showing that neither NF-E2 or EKLf protein is detectable in any of three GATA-1 expressing HeLa cell clones.

Figure 6. Expression of GATA factors in HeLa cells. A. Western blots show production of GATA-2, GATA-3 and GATA-6 in wild-type HeLa cells. B. Gata-6 is produced in HeLa cells at levels equivalent to those seen in the COLO 320 cell line.