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An upstream, DNase I hypersensitive region of the hematopoietic-expressed transcription factor GATA-1 gene confers developmental specificity in transgenic mice

(cis-elements/erythropoiesis)

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ABSTRACT **The transcription factor GATA-1, which is expressed in several hematopoietic lineages and multipotential progenitors, is required for the development of red blood cells and platelets. To identify control elements of the mouse *GATA-1* gene, we analyzed DNase I hypersensitivity of the locus in erythroid chromatin and the expression of *GATA-1*/*Escherichia coli* β-galactosidase (*lacZ*) transgenes in mice. Transgenes with 2.7 kb of promoter sequences are expressed infrequently and only within adult (definitive) erythroid cells. We show that inclusion of an upstream hypersensitive site (HS I) markedly enhances the frequency of expressing transgenic lines and activates expression in primitive erythroid cells. This pattern recapitulates the proper pattern of GATA-1 expression during development. By breeding a *GATA-1*/*lacZ* transgene into a GATA-1$^-$ background, we also have shown that the activation or maintenance of GATA-1 expression does not require the presence of GATA-1 itself, thereby excluding simple models of positive autoregulation. The transgene cassette reported here should be useful in directing expression of foreign sequences at the onset of hematopoiesis in the embryo and may assist in the identification of upstream regulators of the *GATA-1* gene.**

Blood cells arise from pluripotent hematopoietic stem cells (HSCs) (see ref. 1). Although normally quiescent, HSCs generate additional HSCs by self-renewal and also give rise to progenitors committed to various lineages. The site of blood formation shifts during development. In the mouse, hematopoiesis occurs first in the blood islands of the yolk sac (YS) at ≈ embryonic day 7.5 (E7.5), where only primitive (or embryonic) erythrocytes are produced. These cells express embryonic globins and remain nucleated. Within the embryo proper, adult (or definitive) red blood cells, which express adult globins and are enucleate, are produced initially within the fetal liver (FL) at ≈E11. Progenitors of other hematopoietic lineages are detectable by *in vitro* colony assays of YS and FL cells.

Differentiation of hematopoietic lineages relies on the combinatorial action of ubiquitous transcription factors and a set of lineage-restricted factors (1), of which GATA-1 is noteworthy (2, 3). GATA-1 recognizes motifs present in the regulatory regions of virtually all erythroid and megakaryocytic-cell expressed genes (4). With the exception of Sertoli cells in the testis (5, 6), GATA-1 is restricted to the hematopoietic system, where it is present at high levels in erythroid precursors, mast cells, eosinophils, and megakaryocytes (7–10), and at lower level in multipotential progenitors (11, 12). Among the known hematopoietic transcription factors GATA-1 is unique in its capacity to reprogram progenitors to megakaryocytic, erythroid, or eosinophilic differentiation (13, 14).

Gene targeting in embryonic stem cells and mice has established that GATA-1 is essential for maturation of erythroid precursors and megakaryocytes (15–18). GATA-1$^-$ embryos die by ≈E10.5 due to extreme anemia. Primitive proerythroblasts arrest at the proerythroblast stage and undergo apoptosis (16), as do definitive erythroblasts obtained by *in vitro* differentiation of embryonic stem cells (17, 19). In contrast, developmentally retarded GATA-1$^-$ megakaryocytes exhibit hyperproliferation (18).

Defining control elements of the *GATA-1* gene should provide insights into the development of the hematopoietic system. Introduction of *GATA-1* cDNA or genomic constructs driven by 1–2.7 kb of the *GATA-1* gene upstream region into GATA-1$^-$ embryonic stem cells led to incomplete, or infrequent, rescue of differentiation (20, 21). Yet, full rescue is achieved by transfer of a 250-kb yeast artificial chromosome clone containing the locus (L. Lien, M.A.M., and S.H.O., unpublished observations). These findings suggest that regulatory elements outside the immediate 1–2.7 kb of the GATA-1 promoter are critical for high-level GATA-1 expression.

To identify cis-elements for *GATA-1* gene transcription, we have identified DNase I hypersensitive sites (HSs) within the locus and generated transgenic mice harboring genomic sequences linked to the bacterial β-galactosidase (*lacZ*) gene as a reporter. Constructs containing the proximal promoter region plus downstream intron sequences were expressed specifically in definitive hematopoietic cells, but only in a small subset of transgenic lines. Inclusion of an upstream HS led to enhanced, and developmentally appropriate, transgene expression, which was initiated at the onset of YS erythropoiesis. By crossing a *GATA-1*/*lacZ* transgene into a GATA-1$^-$ background, we also have shown that GATA-1 is not required either for activation or maintenance of expression of the *GATA-1* gene despite the presence of high-affinity GATA sites in the promoter postulated to be involved in autoregulation (22–25).

MATERIALS AND METHODS

***GATA-1*/*lacZ* Transgenic Mice.** In all transgenes, a *lacZ* cassette (26) was placed at a *Not*I site engineered at the exon-2 initiator codon of *GATA-1* genomic sequences. The starting *GATA-1* genomic plasmid contains 2.7-kb 5′- and 1-kb 3′-sequences (21). By sequential steps a fragment extending from an intronic *Xba*I site 2 kb 5′ to the *Not*I site was recloned to reconstruct the 5′-portion of the *GATA-1* gene (to 2.7 kb upstream of the IE exon). 3′-Sequences of the *GATA-1* gene

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Abbreviations: YS, yolk sac; FL, fetal liver; HS, hypersensitive site; E, embryonic day; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; UHR, upstream hypersensitive region.

from an exon-3 *Nhe*I site to a 3′-flanking *Hin*dIII site were then added. 5′-*lacZ* and 5′3′-*lacZ* plasmids (see Fig. 1) were created by cloning the *lacZ* cassette into the unique *Not*I site. ≈Seven hundred fifty base pairs extending from the *Nco*I site of exon 2 to the *Nhe*I site in exon 3 of the GATA-1 gene are deleted in the 5′3′-*lacZ* construct. To generate the upstream hypersensitive region (UHR)-5′3′-*lacZ* transgene an ≈8-kb *Sma*I fragment from a phage clone containing IT sequences was used to replace sequences upstream of an intronic *Sma*I site (see Fig. 1). All recombinant procedures were performed by standard methods (27).

Before microinjection, DNA fragments were purified from plasmid sequences by gel electrophoresis. Transgenic mice were generated by pronuclear injection of C57BL/6 × SJL F2 hybrid fertilized eggs for the 5′3′-*lacZ* and 5′-*lacZ* constructs, or CD-1 eggs for the UHR-5′3′-*lacZ* construct. C57BL/6 or CD-1 females were used as recipients for injected embryos and for breeding studies. Expressing lines of 5′-*lacZ* and UHR-5′3′-*lacZ* mice were backcrossed to the opposite CD-1 or C57BL/6 strain to exclude strain differences in LacZ expression (data not shown).

To analyze fetal tissues, timed matings of wild-type CD-1 females with male F_1 transgenic animals were performed. Fetal tissue or YS DNA was used for genotyping by PCR. Tail DNAs were genotyped by DNA slot blots hybridized with an internal 350-bp *Eco*RV/*Cla*I *lacZ* fragment probe or by PCR with primers *lacZ*1: 5′-GCATCGAGCTGGGTAATAAGCGTTGGCAAT-3′, *lacZ*2: 5′-GACACCAGACCAACTGGTAATGGTAGCGAC-3′. Rapsyn primers (Rapsyn1: 5′-AGGACTGGGTGGCTTCCAACTCCCAGACAC-3′, and Rapsyn2: 5′-AGCTTCTCATTGCTGCGCGCCAGGTTCAGG-3′, ref. 28) were used as internal controls. Copy

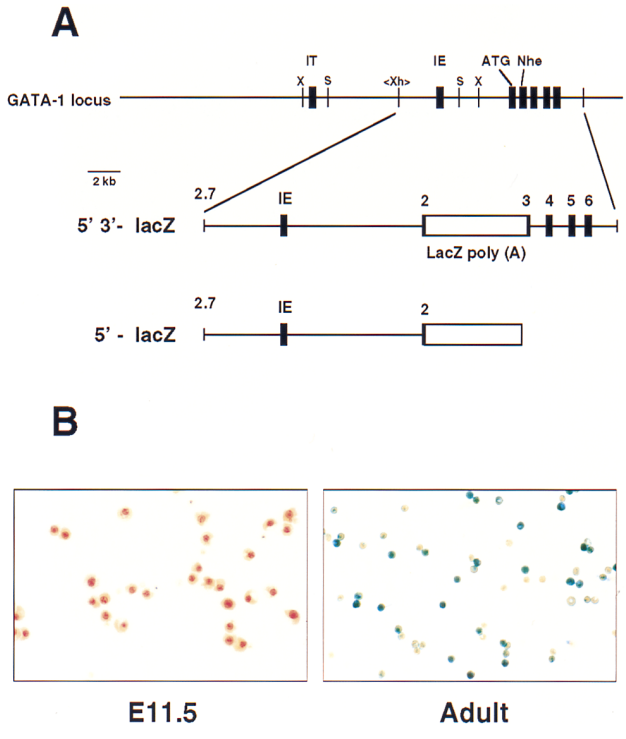

FIG. 1. Transgenes containing 2.7 kb of the GATA-1 promoter are not expressed during primitive erythropoiesis. (*A*) Map of the *GATA-1* locus and the 5′3′-*lacZ* and 5′-*lacZ* transgenes. Exons are boxed and numbered. IT and IE refer to the testis and erythroid promoters, respectively. X, *Xba*I; S, *Sma*I; <Xh>, an artificial *Xho*I site present at the boundary of a phage clone. (*B*) Analysis of β-galactosidase expression in primitive (E11.5 yolk sac) and adult erythrocytes of transgenic line 1379. Neutral red was used as a counterstain after X-Gal staining (100× magnification with oil objective).

number was estimated from PhosphorImager analysis of Southern blots.

β-Galactosidase Assays. Whole-mount and 10-μm frozen sections of glutaraldehyde-fixed, sucrose-saturated tissues were fixed and stained with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) substrate as described (28) except incubations were performed at 37°C overnight. A midline abdominal incision was performed before fixation to facilitate processing of the E14.5 embryo samples. Neutral red was used as counterstain. Bone marrow and blood cell X-Gal staining, and blood cell enzymatic *o*-nitrophenyl β-D-galactoside mouse assays were performed as described (29). Red blood cells were quantitated on a Technicon H1 automated cell counter.

DNase I Hypersensitivity Assays. Mouse erythroleukemia or NIH 3T3 cell nuclei were digested with varying concentrations of DNase I (30). Purified DNA was digested with *Xba*I or *Pvu*II and electrophoresed through 0.7–0.9% agarose gels for Southern blotting. A 220-bp probe spanning the upstream testis exon (IT) was labeled by PCR using IT-exon primers (5′-ACTCTTGCTCTCTTTTGCAG-3′ and 5′-AATCAGGAATGCAACATCTC-3′). A 280-bp *Eco*RI exon-2/3 cDNA probe was used to identify intron HSs.

RESULTS

Transgenes Containing Proximal Promoter Sequences Are Expressed Infrequently and Only During Definitive Erythropoiesis. GATA-1 is expressed from alternative, noncoding first exons, designated IT or IE (Fig. 1*A*). IT is predominantly expressed in the Sertoli cells of the testis (6, 31), whereas IE is primarily active in hematopoietic (22, 31). Initial efforts focused on promoter elements located 1–2.7 kb upstream of the IE exon. Previous work from our laboratory (22, 32) and others (33) demonstrate that these sequences confer a degree of erythroid-specificity in transiently transfected mouse erythroleukemia cells and transgenic mice.

We first tested transgenes including 2.7 kb upstream of IE plus the downstream intron, with or without sequences 3′ of the GATA-1 initiator ATG (constructs 5′3′-*lacZ* and 5′-*lacZ*, respectively; Fig. 1*A*). Founder animals with varying copies of the transgenes were obtained. β-Galactosidase (LacZ) activity was assessed in founders and their progeny at different ages. Few lines expressed detectable LacZ activity above background in adult peripheral red blood cells, bone marrow, or

Table 1. LacZ expression of transgenic mice

Construct	Line	Copy no.	PB	BM	SP	OT
5′3′-*lacZ*	1389	11	−	−	−	−
	1802	15	−	−	−	−
	1803	15	−	−	−	−
	1804	20	−	−	−	−
	1806	2	−	−	−	*
	1809	5	−	−	−	−
	1814	5	−	−	−	−
	1818	10	−	−	−	−
	1819	15	−	−	−	−
	1822	15	+	·	·	−
	1828	2	+	·	·	−
	1834	5	−	−	−	−
5′-*lacZ*	1369	1	+	+	+	−
	1373	7	−	−	−	−
	1374	2	−	−	−	*
	1376	14	−	−	−	−
	1377	5	−	−	−	−
	1379	1	+	+	+	−

PB, peripheral blood; BM, bone marrow; SP, spleen; OT, other tissue (liver, lung, kidney, skeletal muscle); *, expression detected in germ cells of testis. · denotes not above background.

spleen (Table 1). No expression was seen at the YS stage. Expression in adult red blood cells was variable among individual cells. Representative results from transgenic line 1379 are shown in Fig. 1*B*. Apart from rare ectopic staining in germ cells of the testis, LacZ expression, when present, was limited to hematopoietic cells (see Table 1). Taken together, these observations indicate that sequences beyond those contained within the 5′3′-*PacZ* transgene are required for consistent, developmentally appropriate, high-level expression of the *GATA-1 gene.*

DNase I Hypersensitive Mapping Identifies a Candidate, Upstream Element. DNase I hypersensitivity mapping was performed with nuclei of erythroid and nonerythroid (NIH 3T3) cells (34–36). Several regions of hypersensitivity were detected in mouse erythroid, but not 3T3, cell chromatin (Fig. 2). These include a region of strong hypersensitivity upstream of the IE exon (HS I); sites surrounding the IE exon, including the proximal promoter (HS II); and a region within the intron downstream of the IE exon (HS III). HS II and III regions were included in the 5′3′-*lacZ* and 5′-*lacZ* constructs. HS I also was detected in chromatin of freshly isolated FL cells (not shown).

Inclusion of HS I Enhances the Frequency of Transgene Expression and Confers Proper Developmental Specificity. To test the function of the region encompassed by HS I, 4.3 kb of additional 5′-sequences were added to generate the UHR-5′3′-*lacZ* construct (Fig. 3*A*). Twelve independent lines were generated (Table 2). A marked increase in the frequency of transgenic lines expressing in definitive red cells (7/12) was

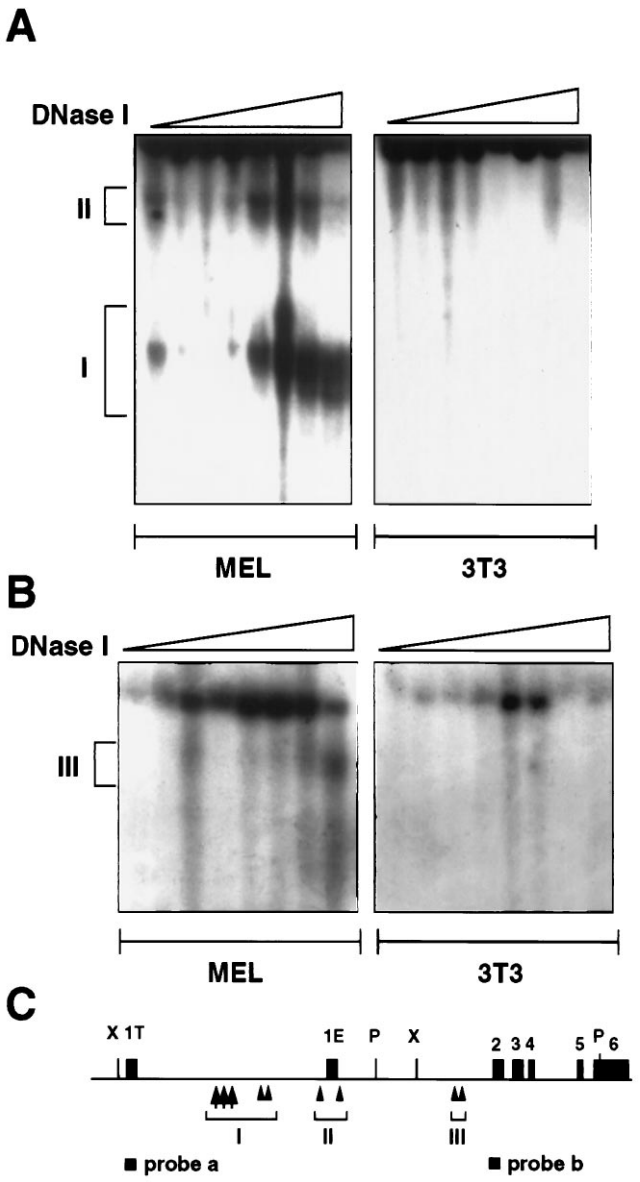

FIG. 2. DNase I HSs within the *GATA-1* locus. (*A*) Map of the mouse *GATA-1* locus. HS I, II, and III are indicated by the arrows. The locations of the probes used for mapping are shown. Sites as in Fig. 1. P, *Pvu*II. (*B*) Nuclei from mouse erythroleukemia cells (MEL) or fibroblasts (3T3) were treated with increasing concentrations of DNase I as indicated by the triangle. Extracted DNA was digested with *Xba*I. The intact *Xba*I fragment is at the top of the autoradiogram. HS I and II are indicated by brackets. (*C*) Identical analysis using a *Pvu*II digest and an exon 2/3 probe. HS III is indicated by the bracket.

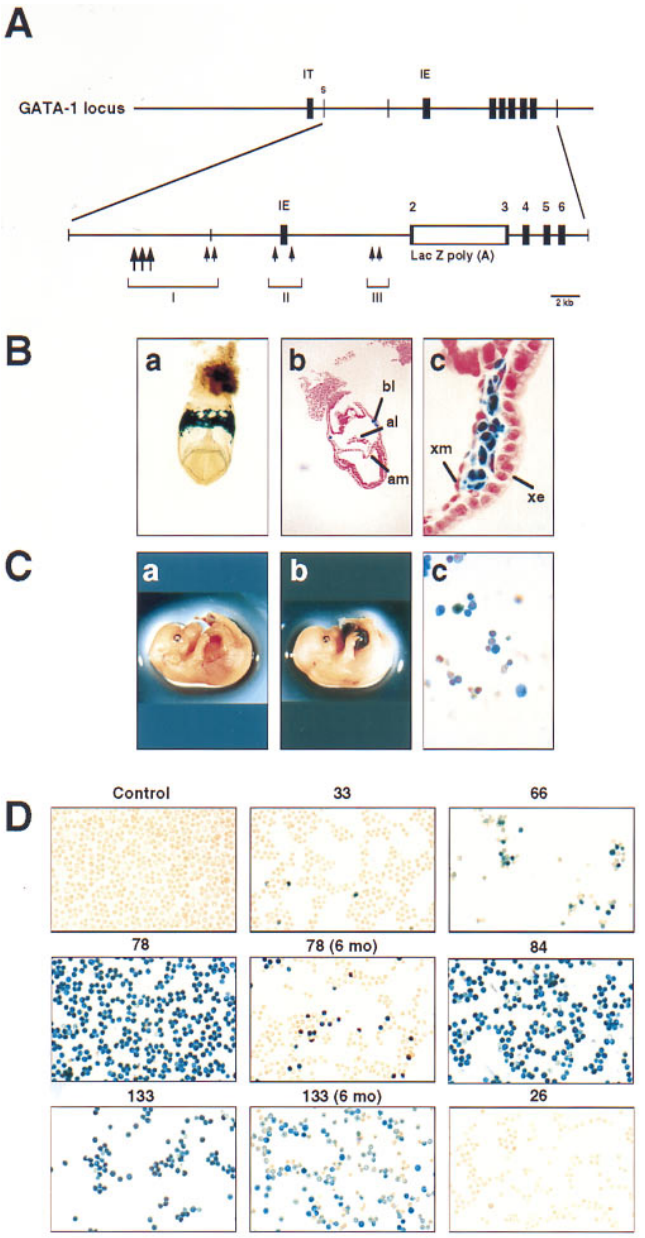

FIG. 3. Expression of β-galactosidase in transgenic lines harboring the upstream HS I region. (*A*) The UHR-5′3′-*lacZ* transgene is shown below the map of the *GATA-1* locus. (*B*) LacZ expression in whole mount (*a*), cross-section (*b*), and blood island (*c*) at E7.5. bl, blood island; al, allantois; am, amnion; xm, extraembryonic mesoderm; xe, extraembryonic endoderm. (*C*) Assay of LacZ expression at the FL stage. The FL is unstained in control mice (*a*) and intensely stained in line 133 transgenic mice (*b*). FL erythroid cells of line 66 mice stain variably (*c*). (*D*) LacZ expression in adult red blood cells of transgenic lines at different ages. Mice were examined at either 4 weeks or 6 months (as indicated).

Table 2. Expression in UHR-5′3′-*lacZ* transgenic mice

Line	Copy no.	PB, %	U/10^{12} RBC	U/10^{12} blue RBC	YS, %
61	1	0	14	na	na
80	1	0	na	na	na
78	1	99	na	na	35
84	1	99	734	840	na
3	2	5	57	810	25
133	2	99	1340	1340	60
66	4	71	92	131	na
67	5	<1	na	na	na
33	10	5	na	na	na
54	20	99	na	na	na
71	20	0	na	na	<1
26	100	0	na	na	0

Peripheral blood (PB) and E10.5–12.5 yolk sac (YS)-derived erythrocytes were assayed for LacZ expression by X-Gal staining. %, percent positive cells; na, not assayed; U, units of β-galactosidase activity assayed with *o*-nitrophenyl β-D-galactoside as substrate; RBC, erythrocytes.

evident (Table 2). Of particular importance, expression was now seen in the YS at E7.5; the vast majority of primitive erythroid cells within blood islands exhibited LacZ staining (Fig. 3*B*, Table 2). The onset of expression closely approximates the appearance of endogenous GATA-1 transcripts at E7.25–7.5 in visceral YS extraembryonic mesoderm (ref. 37, and not shown). Transgene expression persisted at high level in the FL and in peripheral red cells of newborn mice (Fig. 3 *C* and *D*). Thus, inclusion of the upstream region enhances overall transgene expression, while conferring activation specifically within the primitive erythroid cells.

Transgene Expression Is Variegated and Decreases with Age. LacZ-staining of cells was nonuniform. Both the fraction of positive cells and the intensity of staining varied. Although the majority of peripheral red cells of young mice were positive in multiple lines (e.g., nos. 78, 84, 133, and 54), the proportion declined with age (Fig. 3*D*). Staining patterns were consistent for progeny of each transgenic line, indicating that the distribution of positive cells and the variability in staining intensity were intrinsic properties of each line. Age-related silencing is not the result of an irreversible change at the transgene locus. When a 6-month-old transgenic was mated with CD-1 controls, 3-week-old pups expressed at the same level as the initial founder (not shown). Thus, silencing is epigenetic.

To examine apparent differences in staining intensity, we performed enzymatic assays of lysed red cells using *o*-nitrophenyl β-D-galactoside as substrate and calculated β-galactosidase activity per expressing cell (38). The activity per blue cell varied by 10-fold among the lines tested. Lines 3, 84, and 133 expressed similar activities per "on" cell, whereas line 66 expressed at a lower level. Overall, the activity per on cell correlated with the intensity of staining.

Transgenes Containing HS I Also Express Strongly in Megakaryocytes, But Not in Mast Cells, Eosinophils, or Nonhematopoietic Tissues. To examine the specificity of transgene expression further, additional cells and tissues were analyzed. Representative data for line 133 are displayed in Fig. 4. Bone marrow samples revealed intense staining in megakaryocytes, as well as erythroid precursors (Fig. 4 *A* and *B*). Although expression of GATA-1 is silenced as multipotential progenitors commit to the myeloid lineage (11, 39), LacZ staining was readily detected in terminally differentiated granulocytes in transgenic line 133. Whether the presence of LacZ activity in myeloid cells reflects continued expression of the transgene in differentiating myeloid cells or previous expression in progenitors and long-lived, stable *lacZ* mRNA or protein is uncertain. Among lines 3, 61, 66, 80, 84, and 133, nonhematopoietic expression was seen only in germ cells of the testis in line 66. The transgene is inactive in Sertoli cells (Fig. 4*C*).

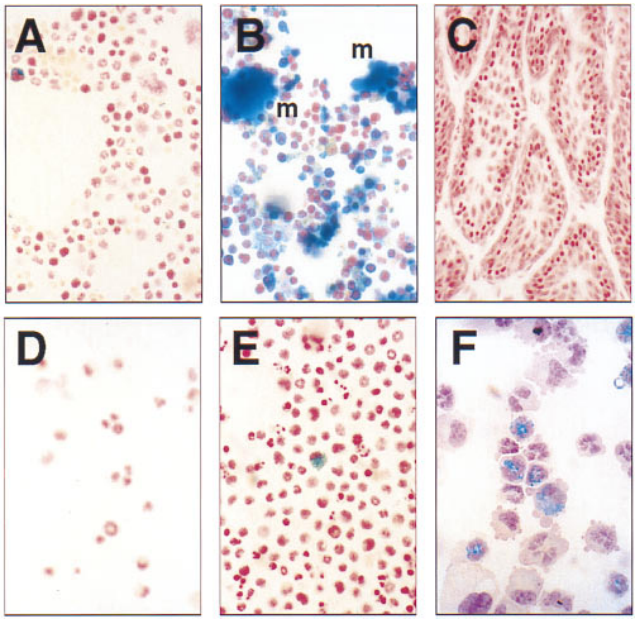

FIG. 4. Tissue-specific expression of UHR-5′3′-*lacZ* transgene. (*A* and *B*) X-Gal-stained bone marrow cells of adult wild-type and transgenic line 133 mice, respectively. m, megakaryocyte. (*C*) Section of testis of 3-week-old male stained with X-Gal reagent. (*D-F*) Bone marrow cells cultured in interleukin 5. (*D* and *E*) X-Gal-stained transgenic line 133 and wild-type, respectively. (*F*) Transgenic line 133 cells stained with Luxol B.

GATA-1 is normally expressed in mast cells and eosinophils (7–9). We performed liquid culture of bone marrow cells in the presence of Epo/KL, interleukin 3, and interleukin 3 + interleukin 5 to obtain erythroid, mast, and eosinophilic cells, respectively. Whereas erythroid cells stained strongly for LacZ activity, mast cells (not shown) and eosinophils failed to stain (Fig. 4 *D* and *E*). Luxol blue staining confirmed the identity of eosinophils (Fig. 4*F*). Thus, expression of the UHR-5′3′-*lacZ* transgene is restricted to a subset of hematopoietic lineages (erythroid, megakaryocytic) known to express high levels of GATA-1.

Activation or Maintenance of *GATA-1* Expression Is Independent of *GATA-1*. *GATA-1* promoters contain high-affinity GATA sites that are required for maximal promoter activity in transient assays (22, 23, 25). Their presence is consistent with the existence of a positive autoregulatory loop serving to maintain the *GATA-1* gene in an on transcriptional state or enhance GATA-1 expression in maturing precursor cells.

By breeding the UHR-5′3′-*lacZ* transgene into a GATA-1^{-} environment, we performed a genetic test of the requirement for GATA-1 in activation or maintenance of gene transcription. Male transgenic mice were interbred with female GATA-$1^{+/-}$ heterozygotes (16). Embryos were sacrificed at E10.5 for analysis of LacZ staining. As shown in Fig. 5, intense staining was evident in YS whole mounts or primitive red cells of embryos carrying the transgene in a GATA-1^{+} background. In a GATA-1^{-} background, YS erythroid cells, which are arrested at the proerythroblast stage due to the absence of GATA-1 (16, 17), are comparably stained. Thus, neither the activation nor the maintenance of *GATA-1* gene expression requires the presence of GATA-1 itself.

DISCUSSION

GATA-1, an essential regulator of erythroid and megakaryocytic differentiation (15–18), is expressed at high level in

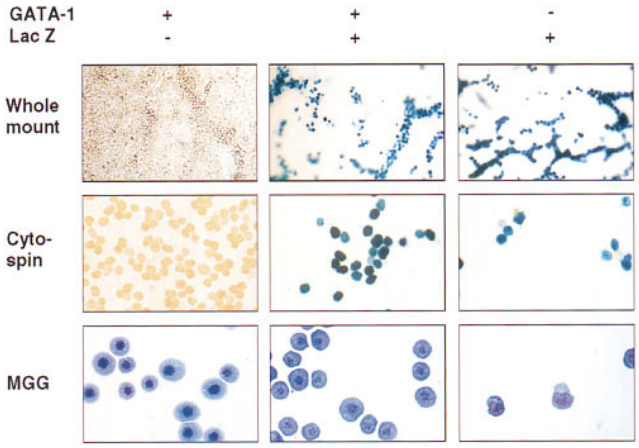

FIG. 5. GATA-1-independent transgene expression. Genotypes are indicated at the top. The presence of a wild-type and null *GATA-1* alleles are indicated by + and −, respectively. The presence of a UHR 5′3′-*lacZ* transgene is indicated by +. (*A–C*) Whole mount preparations of E10.5 YS, stained with X-Gal; (*D–F*) Cytospins of X-Gal-stained erythrocytes. (*G–I*) May–Grunwald–Giemsa-stained erythroid cells. Note the developmental arrest of GATA-1$^-$ cells shown at the bottom right.

several hematopoietic lineages and at a lower level in progenitor cells (11, 12). The mechanisms by which the *GATA-1* gene is activated during development and regulated thereafter are poorly understood. Of particular interest are the upstream factors governing expression of the gene during embryogenesis. The identification of cis-regulatory elements capable of conferring developmentally appropriate expression *in vivo* is a step toward identifying these factors.

Transcription of the *GATA-1* Locus Is Dependent on Multiple Regulatory Elements. DNase I hypersensitivity mapping of erythroid cell chromatin identified at least three regions, HS I, II, and III (Fig. 2). Previous data implicating a high-affinity GATA-site and duplicated CACC-motifs in *GATA-1* promoter function (22) suggest that HS II may reflect protein occupancy of these, and perhaps other, local sequences. While previous experiments demonstrate some erythroid-specificity of the promoter (32, 33), those reported here show that inclusion of sequences encompassing HS I enhances not only the frequency of transgenic lines expressing in definitive erythroid cells, but also activates transgene expression in primitive erythroid cells. This expression profile largely recapitulates the expression pattern of the endogenous *GATA-1* gene during embryogenesis (37). Beyond increasing the proportion of expressing transgenic lines and facilitating gene activation within the YS, the addition of HS I sequences appears to increase the average number of expressing cells, and perhaps also the level at which they express. Thus, the inclusion of the HS I region in transgenes has multiple effects on the activation, maintenance, and level of *GATA-1* gene expression.

It appears likely that sequences in the vicinity of HS I cooperate with those in the proximal promoter, and possibly those surrounding the intron HS III region, to activate and maintain expression in hematopoietic progenitors and their progeny. Sequences within HS III, however, are not obligatory for expression in adult erythroid cells based on their absence in the construct of Skoda *et al.* (32) and from additional transgenic lines we have analyzed lacking this region (not shown). It should be noted that HS III appears to lie in the vicinity of an alternative, infrequently used promoter within the gene (22).

Additional Cis-Regulatory Elements of the *GATA-1* Gene Remain to be Characterized. Despite its inherent specificity, other data indicate that the UHR-5′3′-*lacZ* transgene lacks the full complement of regulatory elements of the *GATA-1* locus. First, the transgene does not exhibit properties characteristic of locus control regions (35). Expression is copy number-independent and subject to position effects (Table 2). While the majority of erythroid cells express in young mice of multiple transgenic lines, variegation is prominent, especially with increasing age. Variegation and age-related silencing are phenomena currently ascribed to the repressive effects of neighboring chromatin on transgenes lacking a full complement of regulatory elements (29, 38, 40–43). Second, although high-level expression of the UHR-5′3′-*lacZ* transgene is observed in erythroid cells and megakaryocytes, expression has not been detected in mast cells and eosinophils above occasional background staining. Failure to express in these lineages reflects either the need for additional sequences to activate transgene expression, or rapid silencing of transgenes within these lineages. Third, targeted mutagenesis indicates redundancy of cis-elements in the *GATA-1* locus (44). ≈Eight kilobases of sequences upstream of −2.7 kb were deleted by homologous recombination (18). While the resulting allele lacks the HS I region, the IT exon, and sequences lying between them, GATA-1 expression is maintained at near-normal level in erythroid precursors (44), but not in megakaryocytes (18). Thus, within the context of the locus *in situ*, sequences encompassing the HS I region are not required for activation of *GATA-1* gene transcription in primitive erythroid cells or subsequent maintenance of expression in erythroid precursors. Presumably, other sequences within the locus compensate for their absence in erythroid cells, but cannot do so in megakaryocytes (18). The location of these additional elements is unknown.

***GATA-1* Is Not Required for Maintenance of *GATA-1* Gene Expression.** UHR-5′3′-*lacZ* transgenic mice are suitable for monitoring trans-regulatory effects on *GATA-1* gene expression. Indirect evidence suggests that GATA-1 may feedback in a positive autoregulatory circuit to maintain or increase expression from its promoter (22–25). By breeding the transgene into a GATA-1$^-$ environment, we have shown that *GATA-1* is not strictly required either for activation or maintenance of GATA-1 expression *in vivo*. While this finding argues against positive autoregulation by GATA-1 in the simplest sense, it does not exclude the positive action of other GATA factors, such as GATA-2 (45, 46), on the *GATA-1* promoter or quantitative effects on promoter activity that may escape detection by LacZ staining. Our findings are analogous to those reported for the myogenin gene where it was shown that myogenin is not required for proper regulation of the myogenin gene (47). In both situations, related family members may overlap in transcriptional functions and, therefore, obscure simple positive autoregulatory mechanisms. As candidate upstream regulators of the *GATA-1* gene are identified, the introduction of the UHR-5′3′-*lacZ* transgene into modified cellular backgrounds will provide a stringent test of genetic interaction.

Potential Applications of a Hematopoietic-Specific Transgene Cassette. Our studies demonstrate that genomic sequences contained within the UHR-5′3′-*lacZ* transgene direct heterologous gene expression at the onset of hematopoiesis in the developing mouse embryo, and thereafter in a subset of hematopoietic lineages. These properties of the transgene cassette could be advantageous in various experiment settings. For example, the cassette might be used to test potential *in vivo* roles of nuclear regulators implicated in hematopoietic lineage selection or cell maturation, express dominant inhibitory versions of molecules believed to serve critical functions in hematopoiesis, or express Cre recombinase to reveal conditional phenotypes (48). Moreover, use of the transgene cassette may permit assessment of the relative position of GATA-1 in a genetic hierarchy with respect to other critical transcriptional regulators within the hematopoietic compartment. Further dissection of the *GATA-1* locus by transgenic

analysis and gene targeting should elucidate the elements essential for gene activation in hematopoietic cells. Ultimately these approaches may define pathways by which growth factors and upstream regulators initiate expression of the *GATA-1* gene in the early embryo.

Recently Onodera *et al.* (49) reported similar studies on the role of upstream sequences in conferring YS expression. Although in their absence Onodera *et al.* failed to detect expression in the limited number of lines tested, we did observe definitive-specific expression in 4 of 18 lines. Thus, in addition to conferring primitive specificity, the upstream region enhances expression in definitive cells.

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