Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 4487–4492, April 1997
Developmental Biology

GATA-1 transcription is controlled by distinct regulatory mechanisms during primitive and definitive erythropoiesis

(enhancer/transgenic mouse)

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Communicated by Bernard Roizman, University of Chicago, Chicago, IL, February 21, 1997 (received for review December 13, 1996)

ABSTRACT **Transcription factor GATA-1 is required for the terminal differentiation of both the primitive and definitive erythroid cell lineages, and yet the regulatory mechanisms of GATA-1 itself are not well understood. To clarify how the GATA-1 gene is transcriptionally controlled *in vivo*, presumptive regulatory regions of the gene were tested by fusion to a reporter gene and then examined in transgenic mice. We found that a transcriptional control element located between −3.9 and −2.6 kb 5′ to the erythroid first exon serves as an activating element and that this sequence alone is sufficient to recapitulate the expression of GATA-1 (but uniquely in primitive erythroid cells). Addition of sequences from the GATA-1 first intron to this upstream element provides a necessary and sufficient condition for complete recapitulation of GATA-1 expression in both primitive and definitive erythroid cells. The first intron element does not possess intrinsic transcriptional activation potential when linked to the GATA-1 gene promoter but rather requires the upstream activating element for its activity. These experiments show that GATA-1 gene expression is regulated by discrete transcriptional control elements during definitive and primitive erythropoiesis: The 5′ element displays properties anticipated for a primitive erythroid cell-specific activating element, and the novel element within the GATA-1 first intron specifically augments this activity in definitive erythroid cells.**

GATA-1 is the first transcription factor shown to be necessary for erythroid-specific gene expression (1, 2). GATA-1 is found in erythroid cells as well as a few other hematopoietic cell lineages (3–5) and also is expressed in Sertoli cells in the testis (6, 7). The prominence of GATA-1 function in erythropoiesis was initially confirmed by the analysis of chimeric mice generated through the use of embryonic stem cells harboring a targeted disruption of GATA-1 gene (8), as well as from *in vitro* differentiation experiments using these same GATA-1-null embryonic stem cells (9–11).

We previously showed that GATA-1 gene transcription in Sertoli cells is directed by a testis-specific promoter/first exon (IT) located ≈8 kb 5′ to the erythroid promoter and first exon (IE) (6). The remaining five exons that encode the GATA-1 protein are used in common in both mature testis and erythroid mRNAs (6). Both the IE and IT proximal promoters were shown to bear duplicated GATA binding sites, and previous transient cotransfection assays suggested that GATA-1 gene expression is regulated by GATA-1 itself or other GATA family transcription factors in both cell types (12–15). However, these cell transfection studies provided only limited physiological insight into the mechanism of GATA-1 gene regulation.

To begin to decipher the transcriptional control mechanisms that regulate GATA-1 using a physiological model, we prepared reporter gene constructs by fusing presumptive regulatory regions of the GATA-1 gene to the *Escherichia coli* β-galactosidase (LacZ) gene and then analyzed reporter gene expression in transgenic mice. These analyses demonstrated that a distinct DNA element, laying between 3.9 and 2.6 kb 5′ to the IE exon (which we refer to as the GATA-1 upstream activating element) directs primitive erythroid cell-specific expression of this gene whereas the expression of GATA-1 in definitive cells requires the first intron in addition to this upstream element. These results indicate that the expression of GATA-1 is controlled by distinct regulatory sequences in primitive and definitive erythroid cells.

MATERIALS AND METHODS

Construction of LacZ Reporters and Generation of Transgenic Mice. Reporter genes were constructed using restriction enzyme sites present in the GATA-1 gene (1, 6, 8) and in the LacZ gene from plasmid pSVβ (CLONTECH). To generate the pIE3.9LacZ plasmid, a *Hin*dIII site was created at the 3′ end of the IE exon using PCR and a primer incorporating a *Hin*dIII site (underlined: 5′-CACAAGCTTGATGCAGAG-GATTCA GCCAC-3′). The fragment between a naturally occurring *Bam*HI site located at 3.9 kb 5′ to exon IE (8) and the *Hin*dIII site was then ligated to pSVβ after removing the simian virus 40 promoter. A pIE2.6LacZ DNA fragment was generated similarly using an *Eco*RI site at −2.6 kb upstream from the IE exon. These constructs both contain a simian virus 40 intron (with splice donor/acceptor sites). To make pIE3.9intLacZ, pIE2.6intLacZ, and pITintIEintLacZ, an *Nco*I site in the second exon (the first coding exon for GATA-1) was used. All of these constructs used the simian virus 40 polyadenylylation signal. After removing vector DNA, the individual constructs were purified with a NACS column (GIBCO/BRL), and 5 to 10 ng/μl of each insert DNA was used for microinjection into mouse oocytes. Transgenic mice were generated using standard strategies (16).

Analysis of the Transgenic Mice and Embryos. For whole mount 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal)

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Abbreviations: IT, testis-specific promoter and first exon; IE, erythroid-specific promoter and first exon; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; dpc, days postcoitus.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AB000965).

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staining, embryos were removed at 7.0, 7.5, and 8.5 days postcoitus (dpc). 0.5 dpc indicates that the dissection was performed at noon of the indicated day after a vaginal plug was found. Embryos were fixed at 4°C for 2 h in 1% formaldehyde, 0.2% glutaraldehyde, and 0.02% Nonidet P-40 in PBS (pH 7.3). After washing with PBS, embryos were incubated at 37°C for 8 h in 2 mM $MgCl_2$, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, and 1 mg/ml X-Gal in PBS. Genomic DNA was purified from the embryos, and integration of the transgenes was verified by PCR. Three primers were used in this analysis: primer 1 corresponds to a sequence in the exon IE, 5′-ACTCGTCATACCACTAAGGT-3′; primer 2 to that in the first intron, 5′-TGTCTCACAACCCTTTCTGTCC-3′; and primer 3 to that in LacZ gene, 5′-GCAACGAAAATCACGTTCTTGTTGG-3′ (see Fig. 1). We also analyzed copy numbers of transgenes by a Southern blot analysis. The copy numbers ranged from 5 to 20, and the variation of copy numbers in the LacZ expression-negative mouse lines was similar to that in the LacZ expression-positive mouse lines.

For tissue section, embryos and adult tissues were fixed and incubated at 4°C overnight in PBS with 20% sucrose. Samples were embedded with Tissue-Tek 4583 OCT compound (Sakura Finetechnical, Tokyo) and rapidly frozen. Frozen sections were stained at 37°C for 8 h with the X-Gal solution. Integration of injected DNAs was verified by PCR using DNA samples purified from the placentae or tissues. Immunostaining using the N6 antibody (7) and anti-murine embryonic hemoglobin (kindly provided by Tadao Atsumi; ref. 17) was performed using the horseradish peroxidase-conjugated secondary antibody and diaminobenzidine as a chromogen after staining with X-Gal at 37°C for 4 h.

Sequence Analysis of the Upstream Activating Region. The 3.9–2.6-kb upstream activating region was subcloned into pBluescript plasmid, and serial deletion mutants were prepared as described (18). Nucleotide sequence was determined using an automated sequencer.

RESULTS

Transgenic Mouse Analysis of GATA-1 Gene Activity Revealed the Presence of an Upstream Activating Element. We generated a number of reporter gene constructs by fusing presumptive regulatory segments of the GATA-1 gene to the LacZ gene (Fig. 1) and then analyzed expression of the reporters in transgenic mice. Of the 266 mice born from foster mothers, 21 mice were determined to be transgenic mice by PCR analysis, and the integrated DNAs were transferred to F1 mice in 15 of the 21 mice. To ensure the reproducibility of the expression profiles, we examined three to four independent, established lines of transgenic mice for each construct. In some instances, we also analyzed transgenic founder embryos directly. As shown in Table 1, the expression of the reporter transgenes was generally quite uniform (i.e., there is very little ectopic expression, and a majority of lines show the same temporal and tissue-specific staining patterns), adding credence to the hypothesis that the regulatory regions identified through this analysis are *bona fide* control elements specifying GATA-1 transcriptional activity.

We first analyzed pIE3.9LacZ, which contains 3.9 kb upstream from the IE exon ligated to the LacZ gene (Fig. 1, construct *a*). In 7.5-dpc embryos, a ring of LacZ expression was observed (Fig. 2*b*, right). This expression was not seen in transgene-negative littermates (Fig. 2*b*, left) nor in normal mouse embryos (data not shown). In 8.5-dpc embryos, this expression had spread throughout the yolk sac (Fig. 2*c*, right; left is a transgene-negative littermate), demonstrating that the

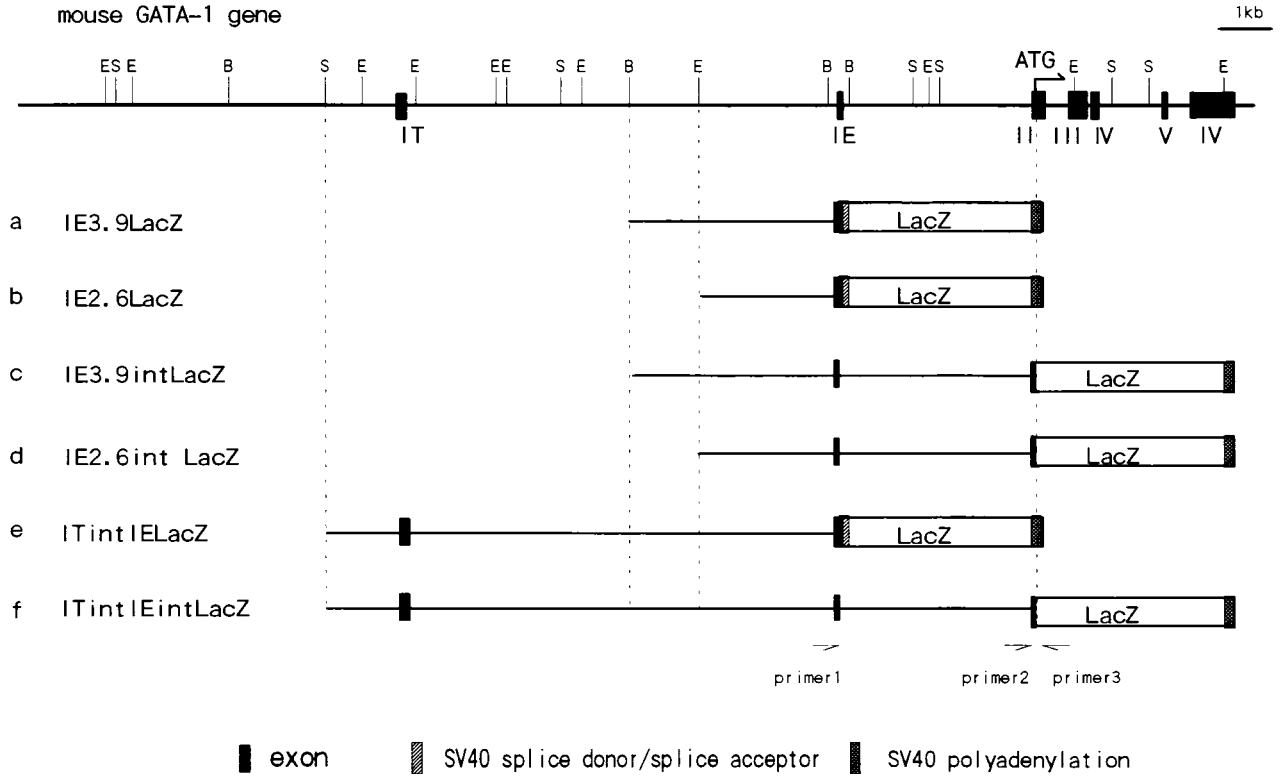

FIG. 1. Construction of reporter genes for promoter function assay and analysis of transgene integration. Mouse GATA-1 locus is shown at the top, where individual exons are depicted as solid boxes. IE and IT indicate the relative position of the erythroid and testis first exons, respectively (6). Abbreviations for the restriction enzyme sites are E, *Eco*RI; B, *Bam*HI; S, *Sac*I. pIE3.9LacZ, pIE2.6LacZ, and pITintIELacZ contain genomic regions ≈3.9 kb, 2.6 kb, or 10 kb 5′ to exon IE fused to the LacZ gene. pIE3.9intLacZ, pIE2.6intLacZ, and pITintIEintLacZ each additionally contain the first intron, where the LacZ gene was fused to the translation initiation codon in the GATA-1 second exon. Locations of the primers used for the screening of the transgenes are also indicated on the diagram.

Table 1. Expression of LacZ in 8.5-dpc yolk sac and 12.5-dpc fetal liver cells of transgenic mouse lines

Constructs	Line	8.5-dpc yolk sac (blue blood island)	12.5-dpc fetal liver, %
a.			
IE3.9LacZ	404	>100	0.9
	932	>100	1.0
	927	0	0
	1	>100	ND
b.			
IE2.6LacZ	547	0	0
	2	0	ND
	5	0	ND
c.			
IE3.9intLacZ	508	>100	24
	515	>100	11
	455	>100	41
	467	>100	0.8
d.			
IE2.6intLacZ	855	0	0
	973	0	0
	803	0	0
e.			
ITintIELacZ	707	>100	0.8
	716	>100	0.8
	12	0	ND
f.			
ITintIEintLacZ	226	>100	42
	114	58	23
	9	ND	20

Numbers of blue blood islands containing X-Gal-positive cells were counted in the 8.5-dpc yolk sacs. Total number of blue blood islands in one typical yolk sac is shown. We also counted 1000 cells in 12.5-dpc fetal liver sections from various transgenic embryos. The percentage of X-Gal-positive blue cells is shown. Lines 1, 2, 5, 9, and 12 were analyzed as F0 embryos. Although a few LacZ-positive cells were observed in the 12.5-dpc fetal liver section of pIE3.9LacZ and pITintIELacZ transgenic mice (see Figs. 3*f* and 5*b*), the same level expression also was observed in nontransgenic embryos. ND, not determined.

expression of GATA-1 occurs concomitantly with the development of blood islands (see below). To define the exact timing of the expression of LacZ, we also analyzed earlier stage embryos of this transgenic mouse line than those having typical yolk sac blood islands. The reporter gene expression commenced in 7.0-dpc embryos (Fig. 2*a*, right), suggesting that GATA-1 gene expression begins at a very early stage of primitive hematopoiesis.

We next examined transgenes bearing 2.6 kb of sequence 5′ to exon IE (pIE2.6LacZ, construct *b* in Fig. 1). We failed to detect any expression of LacZ in the 8.5-dpc yolk sac in any of three independent pIE2.6LacZ transgenic embryos (Table 1). From these results, we deductively revealed the presence of a critical transcriptional activation domain, laying between 3.9 and 2.6 kbp upstream of the IE exon (which we subsequently refer to as the upstream activating element), that regulates GATA-1 gene expression in erythroid cells. The double GATA motifs at ≈−620 nt from major transcription start sites (corresponding to −686 to −672 nt, if the sequence is numbered from the last nucleotide of the exon IE as position −1; ref. 12), which were shown previously to be important for GATA-1 transcriptional control in transfected murine erythroleukemia cells (12), are not capable of independently conferring tissue-specific regulation of GATA-1 in either primitive or definitive erythroid cells *in vivo*.

The Upstream Activating Element Directs Primitive Hematopoietic Cell-Specific Expression of GATA-1. To verify that the β-galactosidase-positive yolk sac cells in the pIE3.9LacZ lines also express GATA-1, embryos bearing this transgene

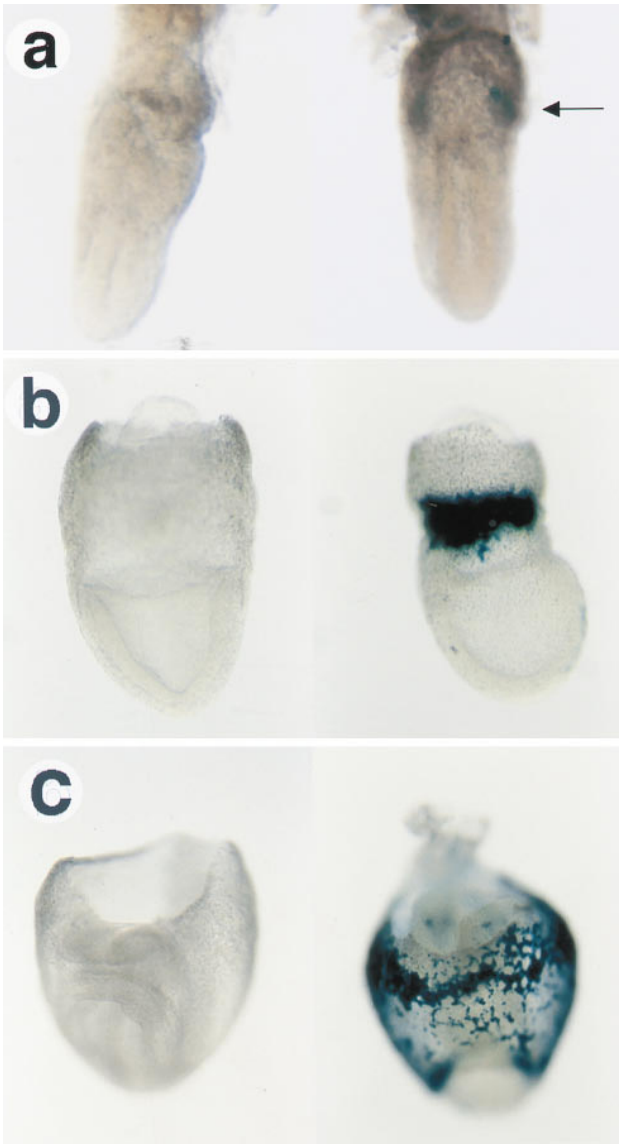

FIG. 2. Primitive erythroid cell-specific expression of LacZ in pIE3.9LacZ transgenic mice. Data from line 404 (see also Table 1) are shown in this figure. Whole mount staining of 7.0- (*a*) and 7.5-dpc (*b*) embryos is shown. LacZ activity is visualized to blue by X-Gal staining (19). Blue staining indicative of β-galactosidase activity is observed in the extraembryonic mesoderm, which will become the yolk sac of a transgenic embryo (right) but not in its transgene-negative littermate (left). The arrow in *a* (right) shows the expression of LacZ in the extraembryonic mesoderm of this early stage embryo. (*c*) The reporter gene expression in an 8.5-dpc embryo; on the right is the transgenic mouse embryo, and on the left is a transgene-negative littermate.

were analyzed histologically. Sections of an 8.5-dpc yolk sac were stained with both X-Gal and anti-GATA-1 mAb N6 (Fig. 3, *a* and *b*). The X-Gal and antibody staining coincided very well in this 8.5-dpc embryo. Of the 823 cells (in 11 blood islands) in the yolk sac of Fig. 3*a*, 97.6% are LacZ-positive blue cells and 94.8% are antibody staining-positive brown cells. These results indicate that the reporter construct contains sufficient sequence information in the 3.9 kb immediately flanking the 5′ end of exon IE to precisely recapitulate GATA-1 gene expression in the primitive erythroid cell population.

We found that, after ≈8.5 dpc, the number of LacZ-positive cells in the yolk sac began to decrease significantly (Table 2), concomitant with the appearance and increase in number of hemoglobinized and more mature primitive erythroid cells

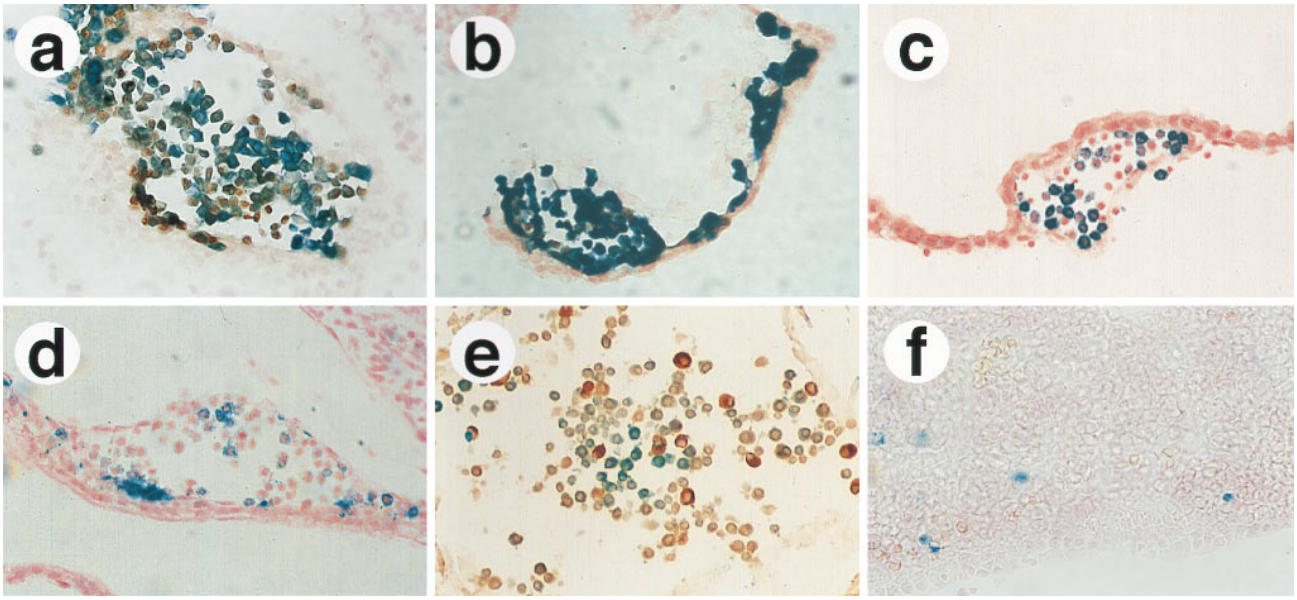

FIG. 3. Progenitor cell-specific expression of GATA-1 in yolk sac primitive erythroid cells. Histological examination of yolk sacs, fetal liver, and circulating blood cells in pIE3.9LacZ transgenic mice (line 404). (*a* and *b*) Sections of 8.5-dpc yolk sacs were doubly stained by X-Gal and anti-GATA-1 mAb N6. Immunohistochemistry was performed with diaminobenzidine as a chromogen (brown color staining). Note that almost all cells in the blood island are stained positively with both X-Gal and the antibody (*a*). Brown color was covered by strong X-Gal staining in *b*. (*c* and *d*) Histological examination of 9.5- and 10.5-dpc yolk sacs. X-Gal-negative cells are increased in the blood islands. (*e*) 9.5-dpc yolk sac was stained with X-Gal and anti-embryonic hemoglobin antibody (17). (*f*) X-Gal staining of 12.5-dpc liver of transgenic mouse embryo. Only a few cells are positively stained to blue. (×128.)

(Fig. 3, *c–e*). Figs. 3 *c* and *d* show X-Gal staining of 9.5- and 10.5-dpc embryo yolk sac blood islands, respectively, and Fig. 3*e* shows X-Gal and anti-embryonic hemoglobin antibody double staining of 9.5-dpc embryo yolk sac blood islands. Thus there appears to be an inverse relationship between the expression of GATA-1 and hemoglobin in the yolk sac blood islands (i.e., in primitive erythroid cells). Less mature, GATA-1-positive primitive erythroid cells did not express hemoglobin whereas more mature, hemoglobinized cells lost GATA-1 expression. These results indicate that primitive erythropoiesis develops synchronously; almost all of the hematopoietic cells in the yolk sac (or its precedent mesodermal tissue) of 7.5- to 8.5-dpc embryos were GATA-1-positive progenitor cells, and these cells then matured into hemoglobinized, GATA-1-negative primitive cells in the yolk sac of 9.5-dpc embryos and thereafter.

Given that the upstream activating element functioned quite efficiently during primitive erythropoiesis, we were surprised to find that the livers of 12.5-dpc transgenic embryos bearing the same pIE3.9LacZ construct displayed only minimal LacZ staining (Fig. 3*f*). The 12.5-dpc fetal liver is the major hematopoietic organ for definitive erythropoiesis at this stage of murine development, so we expected to see many more GATA-1 antibody-positive cells (e.g., see Fig. 4*c*). In addition, we detected only a small number of LacZ-positive erythroid cells in a blood vessel (data not shown) that were (from their nuclear morphology) likely to be primitive erythroid cells. Similarly, we detected only residual LacZ-staining in the adult spleen or bone marrow of these transgenic mice, the intensity and number of which were essentially the same as in nontransgenic littermates (data not shown). These comparative data show that the 3.9-kb region 5′ to exon IE is not sufficient for controlling GATA-1 gene expression in definitive erythroid cells.

Table 2. Decrease of the numbers of X-Gal-positive cells during yolk sac development

Embryo, dpc	Cells counted in blood islands, *n*	LacZ-positive blue cells, *n* (%)
8.5	823	803 (97.6)
9.5	830	529 (63.7)
10.5	1308	443 (33.9)

X-Gal staining-positive cells were counted in 8.5-, 9.5-, and 10.5-dpc embryo yolk sac blood islands.

Regulation of GATA-1 Gene Expression in Definitive Erythroid Cells. Experimental results thus far indicated that definitive erythroid cells use transcription regulatory elements that are different from those used in primitive cells. We therefore initiated a search for the region(s) directing definitive erythroid GATA-1 transcription. We first examined a construct containing almost all of the cloned sequences in the locus surrounding the two promoters; pITintIEintLacZ encompasses a contiguous DNA segment from 1.5 kb 5′ to exon IT (i.e., 10 kb 5′ to exon IE) and terminates 3′ at the translation initiation codon of GATA-1 in exon 2 (Fig. 1, construct *f*).

Transgenic lines generated with this construct displayed strong LacZ expression both in the 8.5-dpc yolk sac (primitive lineage) and in the 12.5-dpc fetal liver (definitive) erythroid cells (Table 1 and Fig. 4, *a* and *b*). The percentages of the blue cells in the fetal liver at 12.5 dpc were 42, 23, and 20% in three independent mouse lines (see Table 1), which were approximately proportional to that of the GATA-1 antibody-positive cells [28.6% (373/1304) of the cells in Fig. 4*c* are antibody staining-positive brown cells]. LacZ activity also was strongly expressed in the red pulp of the adult mouse spleen (Fig. 4*d*) and in bone marrow blood cells (data not shown), both major hematopoietic organs in the adult. Thus, the regulatory region(s) described by this construct are sufficient to facilitate correct temporal and spatial transcription of GATA-1 gene in both primitive and definitive erythroid lineages.

Expression of GATA-1 in Definitive Erythroid Cells Requires Both the Upstream Activating Element and the First Intron. We further dissected plasmid pITintIEintLacZ to identify segments of the locus that specify the expression of GATA-1 in definitive erythroid cells. Several new transgenic lines bearing these constructs were generated (Fig. 1, con-

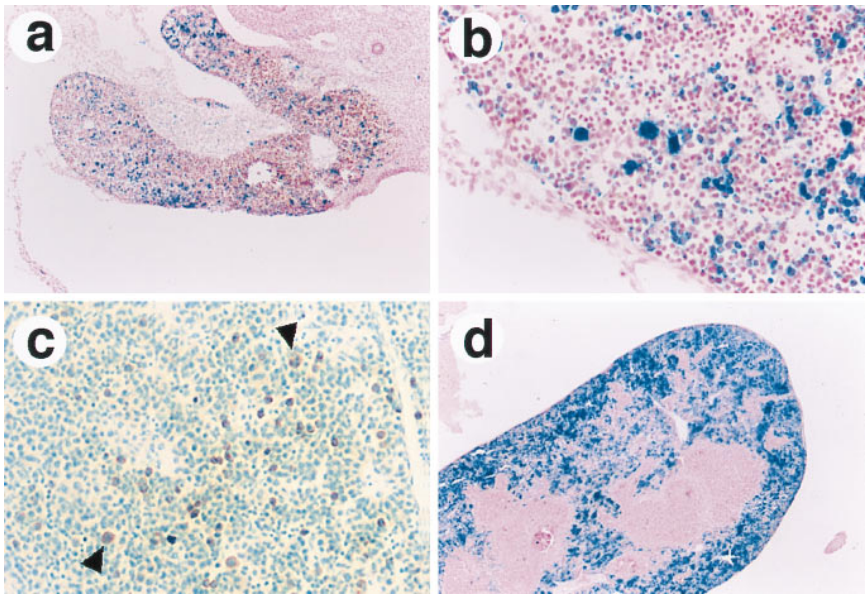

FIG. 4. Definitive erythroid cells express LacZ in pITintIEintLacZ transgenic mice. Data from line 226 (see also Table 1) are shown. (*a* and *b*) Histological examination of a 12.5-dpc fetal liver, where prevalent X-Gal expression was found. (*c*) anti-GATA-1 antibody staining of 12.5-dpc fetal liver. Note that the number of X-Gal-positive cells is comparable to that of the antibody staining-positive cells in the fetal liver. (*d*) X-Gal staining of adult mouse spleen. The expression of LacZ persists in the adult mouse spleen, where many cells in the red pulp show X-Gal staining. (*a* and *d*, ×25; *c*, ×50; and *b*, ×100.)

structs c-e), and the results of their analysis are summarized in Table 1.

The pIE3.9intLacZ transgenic mouse, which harbors the 3.9 kb of 5′ flanking sequence for IE through to the mGATA-1 translational initiation codon (in exon 2), was found to exhibit LacZ expression both in 8.5-dpc yolk sac cells as well as in 12.5-dpc fetal liver hematopoietic cells (Table 1). The expression level of the reporter transgene was comparable to that observed with the parental construct (pITintIEintLacZ; Table 1). In contrast, pIE2.6intLacZ lines, which contain the same sequence information except for the 5′ most 1.3 kb (i.e., missing the upstream activating element), did not show significant LacZ staining activity in either primitive (Fig. 5*a*) or definitive cells (Table 1). Similarly, when the GATA-1 first intron was removed (in pITintIELacZ; Fig. 1, construct *e*), the expression in mice was similar to that of pIE3.9LacZ (Fig. 5*b* and Table 1), indicating that regions further 5′ to the upstream activating element cannot activate transcription from the IE promoter, either alone or in cooperation with upstream activating element. These data show that the upstream element (−2.6 to −3.9 kbp) directs the expression of GATA-1 in primitive erythroid cells and that this element collaborates with sequences within the GATA-1 first intron to confer definitive-specific transcription. The activity of the first intron is unique in that it cannot activate transcription from the IE promoter by itself, in either primitive or definitive erythroid cells (see the results of pITintIELacZ, pIE3.9intLacZ, and pIE2.6intLacZ in Table 1 and Figs. 4 and 5).

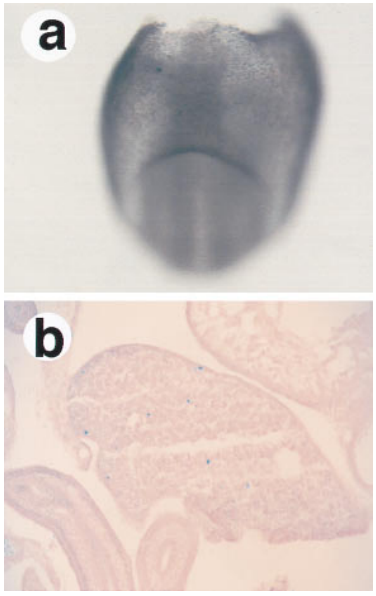

FIG. 5. Expression of GATA-1 in definitive erythroid cells requires both the upstream activating element and the first intron. (*a*) Whole mount X-Gal staining of pIE2.6intLacZ embryo (data from line 855). Note that the intron element alone cannot drive LacZ reporter expression in primitive erythroid cells. (*b*) Histological examination of a 12.5-dpc fetal liver of pITintIELacZ (line 707). (×25.)

The Upstream Activating Element Contains E-Boxes and GATA Motifs. We next determined the nucleotide sequence of the −3.9 to −2.6 kb region harboring the upstream activating element, and this analysis revealed that the actual size of the upstream activating element region was 1183 bp (data not shown). The analysis also revealed the presence of clustered GATA sequences as well as seven E-boxes in this region. In our preliminary analysis, the activating potential of the upstream activating element diminished when 332 bp were deleted from the 5′ end of the element (0/6 F0 embryos examined at 9.5 dpc), but the element was still active when we deleted 100 bp from the 5′ end (3/3 F0 embryos examined at 9.5 dpc). Of interest, a combination of a GATA motif and a E-box resides in this critical 232-bp region. This result suggests that GATA factors and/or erythroid E-box binding transcription factors (such as tal-1/SCL) may be important for the activity of the upstream activating element.

DISCUSSION

Assessment of lineage-specific transcription factor activities leads naturally to the questions of which genes are regulated by these factors and also to how the regulation of the transcription factors themselves is achieved. GATA-1 activity was originally defined as a DNA sequence and then as a protein

binding site, precisely because it was found in virtually every erythroid gene regulatory element that had been defined (reviewed in refs. 20–22), and thus greater interest has focused on the mechanisms that might control GATA-1 activity. Precise regulation of tissue-restricted transcription factors must be essential for the development and maintenance of distinct cell lineages, and although the activity of the GATA-1 protein has been examined in many studies, the nature of its transcriptional regulation has been only roughly outlined. Here we present *in vivo* evidence that GATA-1 is also scrupulously regulated at the level of transcription and further that its transcription in primitive and definitive erythroid progenitor cells is differentially regulated.

One effective strategy by which different lineages of cells might ensure strict transcriptional regulation is to use multiple promoters, and such cases already have been identified for several erythroid transcription factor genes (e.g., see ref. 23) including GATA-1 (6). In addition to alternative promoter use, we have shown here that a distinct regulatory region in the GATA-1 first intron is required for the expression of GATA-1 in definitive erythroid cells, but its effect must be superimposed on the activity of a more classic upstream activating element, which is required for the expression of GATA-1 in primitive cells. The use of multiple regulatory elements, in addition to differential promoter utilization, is another strategy cells appear to have adopted for the regulation of specialized expression of lineage-restricted transcription factors.

The function of the definitive element in the GATA-1 first intron seems to be unlike any other that has been described in the literature because the intron can convert the upstream element, which confers primitive-specific transcription, to one that is now active in definitive erythroid cells. The intron element alone cannot activate transcription from the IE promoter, and its presence also appears to be entirely dispensable for GATA-1 gene function in primitive erythroid cells. These data therefore show that the intron does not harbor activity that one might ascribe to a classical enhancer. One could propose several alternative explanations for how this intron activity might convert the upstream activating element to a definitive erythroid-specific function, and therefore the mechanisms that might confer this GATA-1 first intron function remain to be elucidated.

The difference in the regulation of GATA-1 gene expression in primitive and definitive erythroid cells is probably functionally significant. The definitive hematopoietic stem cell arises from the aorta-gonad-mesonephros region of the embryo, which is different from the origin of primitive hematopoietic cells (i.e., mesoderm). The existence of a reverse hierarchy during primitive and definitive erythropoiesis has been proposed (24–26), and the observed regulatory profiles of the GATA-1 gene seem to be compatible with the proposition. The appearance of colony-forming unit-culture cells is earlier than that of both colony-forming unit-spleen and the long term repopulating hematopoietic stem cells in the yolk sac. GATA-1 expression begins at 7.0 dpc in yolk sac hematopoietic cells (Fig. 2), which corresponds well to the time of appearance of the earliest primitive hematopoietic cells (27). The primitive cells expand through differentiation into mature primitive erythroid lineage cells, presumably under the immediate influence of GATA-1, and primitive erythroid cells become available for the commencement of blood circulation at ≈9.5 dpc.

In definitive hematopoiesis, long term repopulating hematopoietic stem cells differentiate into multiple hematopoietic lineages. The expression of GATA-1 in the adult mouse bone marrow is first detected in Sca-1$^-$/c-kit$^+$/Lineage$^-$ cells, which are distinguishably differentiated from the most immature, pluripotent hematopoietic stem cell blood compartment (Sca-1$^+$/c-kit$^+$/Lineage$^-$) (28). GATA-1 is expressed only in a limited number of lineages or stages during definitive hematopoiesis. The acquisition of an additional cis-regulatory element of GATA-1, the intron element, may therefore be an important event for definitive hematopoiesis because the newly emergent, and distinct, definitive erythroid lineage can now differentiate under the direct or indirect control of this new regulatory region.

We thank Drs. K. Araki, T. Atumi, K. Igarashi, N. Kajiwara, N. Kasai, K-C. Lim, T. Ono, N. Suwabe, K. Todokoro, F. Sugiyama, and K. Yagami for help and discussion. This work was supported by Grants-in-Aid from the Ministry of Education, Science, Culture, and Sports, the Japanese Society for Promotion of Sciences (International Collaborative Research and RFTF96I00202), and the Uehara Memorial Foundation.

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