T-cell Expression of the Human GATA-3 Gene Is Regulated by a Non-lineage-specific Silencer*

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The GATA-3 transcription factor is required for development of the T-cell lineage and Th2 cytokine gene expression in CD4 T-cells. We have mapped the DNase-I-hypersensitive (HS) regions of the human GATA-3 gene in T-cells and non-T-cells and studied their transcriptional activities. HS I–III, located 5′ from the transcriptional initiation site, were found in hematopoietic and non-hematopoietic cells, whereas HS IV–VII, located 3′ from the transcriptional start site, were exclusively observed in T-cells. Among these hypersensitive sites, two transcriptional control elements were found, one in the first intron of the GATA-3 gene and the other between 8.3 and 5.9 kilobases 5′ from the GATA-3 transcriptional initiation site. The first intron acted as a strong transcriptional activator in a position-dependent manner and with no cell-type specificity. The upstream regulatory element could confer T-cell specificity to the GATA-3 promoter activity, and analysis of this region revealed a 707-base pair silencer that drastically inhibits GATA-3 promoter activity in non-T-cells. Two CAGGTG E-boxes, located at the 5′- and 3′-ends of the silencer, were necessary for this silencer activity. The 3′-CAGGTG E-box could bind USF proteins, the ubiquitous repressor ZEB, or the basic helix-loop-helix proteins E2A and HEB, and we showed that a competition between ZEB and E2A/HEB proteins is involved in the silencer activity.

Lineage commitment and differentiation of multipotent hematopoietic stem cells occur throughout life and are mostly regulated at the transcriptional level (1). Multiple studies have now shown that lineage-restricted expression of a subset of transcription factors is essential to achieve proper development of all the hematopoietic lineages, and therefore, the knowledge of the mechanisms involved in the regulation of the expression of these lineage-restricted transcription factors is of considerable importance for further understanding of hematopoiesis.

Transcription factors of the GATA family are related by their conserved zinc-finger motif that binds to the consensus DNA sequence 5′-(AT)GATA(A/G)-3′ (2). Among the GATA factors, GATA-1, -2, and -3 are necessary for hematopoiesis, as gene disruption of any one of these factors results in major hematopoietic defects (3–6). GATA-1, -2, and -3 display different lineage-restricted patterns of expression in hematopoietic cells. GATA-1 is abundant in the erythroid, mastocytic, and megakaryocytic lineages and is also present at a lower level in multipotential progenitors (7–9); GATA-2 is mostly expressed in uncommitted hematopoietic progenitors, immature erythroid cells, and proliferating mast cells (10); and GATA-3 is expressed in very immature hematopoietic progenitors and then only in the T-cell and natural killer cell lineages (11, 12). GATA-3 was first shown to be abundantly expressed in T-lymphocytes, natural killer cells, and embryonic brain (13, 14). More detailed studies have shown that GATA-3 gene expression occurs in numerous sites during development: placenta, kidney, and adrenal gland; the embryonic central and peripheral nervous systems; and embryonic liver and thymus (14). Contrasting with this wide expression during development, GATA-3 mRNAs are mostly detected in thymocytes and T-lymphocytes and in the central nervous system in the adult. This gene expression pattern, regulated during development and cellular differentiation, might be mediated by a complex array of cis-acting elements, as shown in the regulation of transcription factor genes in invertebrates (15). As for hematopoiesis, an extinction of GATA-3 gene expression in stable cell hybrids formed by fusion of cell lines representing the erythroidic and the T-lymphocytic lineages indicated that GATA-3 might be repressed in hematopoietic non-T-cells (16), a kind of regulation already shown for the CD4 gene, another T-cell specific gene (17).

The human, mouse, and chicken GATA-3 genes have been cloned, and sequence analysis of their promoters revealed that they are embedded within a CpG island and share structural features often found in promoters of housekeeping genes (14, 18, 19). Transfection experiments of reporter genes controlled by the mouse or chicken GATA-3 promoter have failed to show any appropriate T-cell-regulated expression, which indicates that the GATA-3 regulatory elements lie 5′ and/or 3′ from the GATA-3 promoter (14, 19). To understand the transcriptional controls that regulate GATA-3 gene expression in T-cells, we have mapped the DNase-I-hypersensitive (HS) regions of the human GATA-3 gene in T-cells and in hematopoietic non-T-cells, and we have studied the role of these regions in GATA-3 gene expression in T-cells.

MATERIALS AND METHODS

Nuclei Preparation and DNase-I Treatment

Approximately 10⁸ cells were washed twice in phosphate-buffered saline; resuspended in 20 ml of homogenization buffer (10 mM Tris (pH 7.4), 15 mM NaCl, 60 mM KCl, 1 mM EDTA, 0.1 mM EGTA, 0.15 mM spermine, 0.5 mM spermidine, and 5% sucrose) containing 0.05% (Jurkat), 0.2% (K562), or 0.6% (HeLa) Nonidet P-40; and broken by five strokes of a Dounce homogenizer. Nuclei were purified by centrifugation over a sucrose gradient (10% sucrose in homogenization buffer), washed twice in wash buffer (15 mM Tris (pH 7.4), 15 mM NaCl, 60 mM

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† The abbreviations used are: HS, hypersensitive; bp, base pair; kb, kilobase(s); CAT, chloramphenical acetyltransferase.
**FIG. 1.** A and B, mapping of the DNase-I HS sites in the human GATA-3 locus after BgII-EcoRV or SalI digestion, respectively. The map shows the human GATA-3 gene, the BgII-ClaI and SspI-SstI probes used, and the resulting fragments observed after DNase-I digestion together with the 12-kb BgII-EcoRV or the 17-kb SalI-SalI germ line fragment. HS sites are depicted by arrows. HS V, located at the beginning of intron 3, could not be distinguished from the germ line fragment in the SalI digestion. DNA was extracted from nuclei treated with increasing amounts of DNase-I, digested with BgII and EcoRV or SalI, electrophoresed, blotted, and hybridized with the indicated probe. Jurkat cells (a T-cell line)
KCI, 0.15 mM spermine, 0.5 mM spermidine, and 10% sucrose), and subjected to increasing concentrations of DNase-I (0.1–15 mg/ml; Worthington) in wash buffer plus 1 mM MgCl₂ for 10 min at 37 °C. The reaction was stopped by the addition of protease K (0.1 mg/ml final concentration), SDS (1% final concentration), and EDTA (10 mM final concentration).

**DNA Extraction and Southern Hybridization**

DNA was extracted by proteinase K digestion (0.1 or 0.2 mg/ml) at 56 °C overnight, followed by phenol/chloroform extraction and ethanol precipitation. 10 μg of DNA were digested to completion with the indicated restriction enzymes, electrophoresed on 0.8% agarose gels, and transferred to nitrocellulose membranes (Hybond C Extra, Amer sham Pharmacia Biotech) by Southern blotting. Hybridization was performed with random-primed 32P-labeled probes at 65 °C in 5× SSC (0.6 M NaCl and 0.06 M sodium citrate [pH 7]), 1× Denhardt’s solution, 20 mM NaPO₄ (pH 6.7), and 10% dextran sulfate. Highest stringency washes consisted of 0.1× SSC and 0.1% SDS at 65 °C. The genomic probes used for the DNase-I studies were a 582-bp BglI-II-SstI fragment and a 441-bp SspI-I-SalI fragment.

**Construction of Plasmids**

Constructs Used to Delimit the Human GATA-3 Promoter—The –96/ +598 DNA fragment was cloned from a cosmid that contained the human GATA-3 gene by BamHI-BstEII digestion, followed by electrophoresis and fragment purification. The –96/+44 DNA fragment was obtained by an XmnI digest of the BamHI-BstEII fragment, followed by purification of the BamHI-XmnI fragment. Mutants of the –96/+598 DNA fragment were obtained by double restriction digests of the –96/+598 DNA fragment, followed by fill in with Klenow polymerase and blunt-end ligation. The constructs used for orientation and position dependence of the 3′-activating element were obtained by cloning a Real-BstEII DNA fragment containing the 3′-activating element 5′ or 3′ from the –96/+44 DNA fragment.

Constructs Used to Characterize a Human GATA-3 Gene-regulating Element—A human placental DNA library (CLONTECH) was screened with a 5′-probe obtained from a cosmid previously cloned, and a phage containing 12 kb of DNA 5′ from the human GATA-3 transcriptional initiation site was isolated. A 2.4-kb BamHI-BamHI DNA fragment containing HS I and HS II (–8300/–5990 fragment) was subcloned into pBSK, and the various constructs shown in this study were obtained by subsequent digests of the BamHI-BamHI DNA fragment. All point mutations were obtained by polymerase chain reaction and subsequent cloning. All the constructs were finally cloned 5′ from the CAT reporter gene using the pBLCAT-3 vector (20). All these constructs were sequenced before use. Sequence analysis was performed using a Taq DyeDex Terminator Cycle Sequencing kit (Applied Biosystems, Inc., Foster City, CA) and an automatic sequencer (Applied Biosystems Model 373A).

Construct Used to Overexpress ZEB—ZEB cDNA was cloned by reverse transcription-polymerase chain reaction using oligonucleotides that encompass the ATG initiation codon and the TAA stop codon. The polymerase chain reaction product was sequenced and cloned into the pCEC vector (21), where the inserted cDNA is driven by the SV40 promoter and enhancer.

**Cell Culture and Transfection**

Human cell lines were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (HeLa) or in RPMI 1640 medium supplemented with 10% fetal calf serum (Jurkat and K562), L-glutamine, penicillin, and streptomycin (Life Technologies, Inc.). For transient transfection, 10⁷ cells were transfected by electroporation with 20 μg of linearized plasmid together with 1 μg of plasmid containing the SV40 promoter-driven neomycin- phosphotransferase gene. After selection on G418, three independent pools of transfected cells were assayed for CAT activity.

**Nuclear Extracts and DNA Binding Assays**

Nuclear extracts were prepared from HeLa and Jurkat cell lines (24), and DNA binding assays were performed essentially as described (25). Poly(dI-dC) was used as nonspecific competitor, and in competition assays, 50 ng of unlabeled competitor DNA was preincubated with the nuclear extract for 5 min before the addition of the labeled probe (0.5 ng). The μE5 and MEF1m oligonucleotides used have been previously described (26), and the 3′-CAGGTG E-box oligonucleotide is 5′-AGCT-TTTTACAGTTGTCCTCTA-3′. Antibodies against HEB and E2A proteins were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against human USF1 and USF2 were a kind gift from Dr. Michel Raymondjean (INSERM U.129, Paris, France), and anti-ZEB antiserum was a gift from Dr. H. Kondoh (Osaka University, Osaka, Japan).

**RESULTS**

**Mapping of the DNase-I-hypersensitive Sites of the Human GATA-3 Locus**—Hypersensitivity of chromatin to DNase-I digestion has been used to identify regulatory elements of numerous genes (27, 28). To determine which regions of the human GATA-3 locus might be implicated in the regulation of its expression in T-cells, we mapped the DNase-I HS sites in 35 kb of human genomic DNA encompassing the human GATA-3 transcription unit in a T-cell line (Jurkat) that expresses human GATA-3 and in a non-hematopoietic cell line (HeLa) and an erythroidic cell line (K562) that do not express human GATA-3. Fig. 1A shows the mapping of DNase-I HS sites after EcoRV-BgIII digestion and hybridization with a 5′-BglII-ClaI probe. In addition to the 12-kb germ line fragment, two fragments of 7 and 2.5 kb were observed exclusively in the Jurkat cell line, and a fragment of 1.1 kb was detected in the three cell lines studied. The 2.5-kb fragment results from DNase digestion around the transcriptional initiation site of the human GATA-3 gene; the 7-kb fragment located an HS site in intron 3, and the 1.1-kb fragment identified a region located 1.5 kb 5′ from the transcriptional initiation site of the human GATA-3 gene. Using SspI digestion and a 3′-SspI-SspI probe, we then mapped two HS sites, located between exons 5 and 6 and found only in Jurkat T-cells (Fig. 1B). Using other digests, we finally mapped the seven HS sites that are shown in Fig. 1C. Interestingly, the 5′-HS sites were found in all the cell lines tested, whereas the 3′-HS sites were found only in the Jurkat cell line.

**Human GATA-3 Contains a Minimal Promoter That Extends in the First Intron**—To look for any regulatory function of the HS sites previously mapped, we first delimited the human GATA-3 minimal promoter. A segment of the human GATA-3 gene encompassing the presumptive minimal promoter, from nucleotides –96 to +44, was first studied (Fig. 2A). After transient transfection into a T-cell line (Jurkat) and into two cell lines that do not express human GATA-3 (HeLa and K562), we detected a weak transcriptional activity that did not display any cell-type specificity (Fig. 2A). By primer extension, we showed that the transfected constructs were correctly initiated in the three cell lines (data not shown). The addition of 5′-
FIG. 2. A, characterization of the human GATA-3 promoter. The CAT reporter gene was transcriptionally directed by the −96/+44 or −96/+598 DNA fragment. These constructs were transfected into a T-cell line (Jurkat; □) that expresses GATA-3 and into a non-hematopoietic cell line (HeLa; □) and an erythrocytic cell line (K562; □) that do not express GATA-3. After normalization, CAT activities were detected and quantified using a PhosphorImager. The values represent the average of at least three independent transfections, and 1 represents the CAT activity obtained with the empty pBL-CAT-3 vector. The bent arrows indicate the human GATA-3 transcriptional initiation site. The white boxes indicate the first exon of the human GATA-3 gene. B, an activating cis-acting sequence is located within the first intron of the human GATA-3 gene. Sequential 3′-deletions of the −96/+598 human GATA-3 DNA fragment were cloned 5′ from the CAT reporter gene as indicated under "Materials and Methods" and transfected in the three cell lines previously described. C, the sequence located at the 3′-end of the first human GATA-3 intron acts in a position-dependent manner. The +208/+598 3′-activating sequence was cloned in both orientations upstream or downstream from the −96/+44 DNA fragment, and the resulting constructs were transfected in the three cell lines studied. The indicated values represent the average of three independent transfections.
sequence (up to −2500) did not change the transcriptional activity of the −96/+44 DNA fragment or bring any T-cell specificity to the constructs used (data not shown). However, the addition of 554 nucleotides located 3′ from the −94/+44 DNA fragment resulted in a 6–10-fold enhancement of transcriptional activity in the three cell lines (Fig. 2A). This 554-nucleotide fragment contained both the first exon and most of the first intron of the human GATA-3 gene and, together with the −96/+44 DNA fragment, defined the −96/+598 human GATA-3 minimal promoter. To delimit the regions involved in the activity of this promoter, we subjected this fragment to 3′-deletion analysis. As shown in Fig. 2B, this analysis defined a 123-bp DNA fragment, located at the end of the first intron, as necessary for efficient activity of the human GATA-3 promoter. We then looked for any position dependence of this 3′-element. A 390-bp DNA fragment containing the human GATA-3 promoter 3′-element was cloned in both orientations 5′ or 3′ from the −96/+44 DNA fragment (Fig. 2C). Only the constructs that contained the 390-bp DNA fragment 3′ from the −96/+44 DNA fragment were transcriptionally active in the three cell lines tested, indicating that the 3′-element acted as a strong transcriptional activator in a position-dependent manner and with no cell-type specificity.

Characterization of a DNA Fragment That Confers T-cell Specificity to the Human GATA-3 Minimal Promoter—All the DNA fragments that encompassed the different HS sites mapped in the human GATA-3 3′-HS sites, we performed deletion analysis to study, by stable transfections, the three 5′-HS sites (Fig. 3). The 8.3-kb DNA fragment that contained all the 5′-HS sites displayed a T-cell specificity. The 6.5-kb DNA fragment containing only HS II and HS III showed a higher transcriptional activity in Jurkat cells than in HeLa or K562 cells, but the difference between these three cell lines was smaller than that observed with the 8.3-kb DNA fragment, as the transcriptional activity of this 6.5-kb DNA fragment was present in HeLa and K562 cells (Fig. 3). The 3-kb DNA fragment that contained only HS III displayed no cell-type specificity and was as active as the human GATA-3 minimal promoter in all three cell lines (Fig. 3). To demonstrate that HS I and HS II were sufficient for T-cell specificity, a 2.1-kb DNA fragment that contained these two HS sites was cloned 5′ from the human GATA-3 minimal promoter, and this construct was stably transfected into Jurkat, K562, and HeLa cells. As shown in Fig. 3, this 2.1-kb DNA fragment conferred T-cell specificity to the human GATA-3 minimal promoter, indicating that HS I and HS II were necessary and sufficient for T-cell specificity of the human GATA-3 minimal promoter in the assay we used.

HS I Acts as a Strong Silencer in Non-T-cells—To characterize the function of HS I and HS II, a 966-bp DNA fragment (−8025 to −7059) containing only HS I was cloned 5′ from the human GATA-3 minimal promoter, and the resulting construct was stably transfected into HeLa, K562, and Jurkat cell lines. This fragment repressed human GATA-3 promoter activity in the HeLa and K562 cell lines, but not in the Jurkat T-cell line. The repression level was identical to the one obtained with the HS I-HS II DNA fragment (Fig. 4), and interestingly, this silencer did not reduce the transcriptional activity of the human
GATA-3 minimal promoter in T-cells (data not shown). The orientation dependence of this negative regulatory element was tested by inserting the 966-bp DNA fragment in reverse orientation 5' from the human GATA-3 promoter, and indeed, the transcriptional inhibition obtained with this construct was the same, indicating that this silencer was orientation-independent (Fig. 4). We then cloned a 1.1-kb DNA fragment (−7059 to −5900) containing only HS II 5' from the human GATA-3 minimal promoter and stably transfected the resulting construct into HeLa, K562, and Jurkat cells. We obtained a 5–6-fold decrease in the transcriptional repression in HeLa and K562 cells, and we got a 2–3-fold increase in the transcriptional activity of the human GATA-3 promoter in Jurkat cells (Fig. 4). These results indicate that HS I contains a major regulatory element that confers T-cell specificity to the human GATA-3 minimal promoter. We thus performed a detailed analysis of this silencer.

A 5'- and a 3'-Element Are Necessary for HS I Silencing—5'- and 3'-deletion analysis of this silencer revealed two cis-acting elements that were necessary for efficient silencing of the human GATA-3 promoter activity. The 5'-element was located between −7828 and −7746, and the 3'-element was located between −7197 and −7121 (Fig. 5). This analysis was done by stable transfection, and we next examined whether these elements were sufficient for efficient repression. We cloned the various deleted regions 5' from the human GATA-3 minimal promoter and transfected these constructs into HeLa or Jurkat cells, but never obtained any efficient repression of the human GATA-3 promoter (data not shown), suggesting that the silencer identified requires multiple elements to be functional. The sequence of the 707-bp DNA fragment (−7828 to −7121) containing HS I is shown in Fig. 6. The 3'-DNA fragment necessary for efficient repression in non-T-cells contained a YY1-binding site adjacent to a CAGGTG E-box and a TC-CTCCT motif already shown to be required for neuronal expression of the zebrafish gata-2 gene (29), and the 5'-DNA fragment characterized also contained a CAGGTG E-box. The presence of this same motif in the 5'- and 3'-regions of the silencer prompted us to analyze its function.

The Two CAGGTG E-boxes Are Necessary for HS I Silencing活性—As the −7828/−7121 DNA fragment has the same transcriptional activity as the initial −8025/−7059 repressor, the role of the two CAGGTG E-boxes in HS I silencing activity was tested in this DNA fragment beginning with the 5'-region. Mutations that deleted or disrupted the 3'-CAGGTG E-box were shown by gel-shift analysis to prevent binding of any protein (data not shown), and stable transfections of the resulting constructs showed that they were completely unable to silence the human GATA-3 promoter activity in non-T-cells and did not modify this promoter activity in T-cells (Fig. 7A). A similar mutation was performed on the 5'-CAGGTG E-box, and stable transfections of the mutated silencer showed that it could not repress the human GATA-3 promoter activity in non-T-cells (Fig. 7B). These results indicate that the HS I silencing activity needs these two CAGGTG E-boxes.

The 3'-CAGGTG E-box Could Bind ZEB, USF, and E2A Proteins—To characterize the proteins that can bind the 3'-CAGGTG E-box, we first performed gel-shift analysis using Jurkat or HeLa nuclear extract. As shown in Fig. 8A, two complexes (C1 and C2) were obtained with Jurkat or HeLa nuclear extract, whereas a third complex (C3) was obtained only with Jurkat nuclear extract. To define the proteins present in these three complexes, we first used competition with oligonucleotides known to bind E2A or related proteins (μE5 oligonucleotide) or the ubiquitous ZEB repressor (MEF1m oligonucleotide) (26). These experiments showed that the C1 complex had the same migration as a ZEB complex (Fig. 8B, lanes 4 and 7) and that the 3'-CAGGTG E-box oligonucleotide efficiently competed the ZEB binding on the MEF1m oligonucleotide (lanes 7 and 8). The C3 complex migrated like the E2A complex bound on the μE5 oligonucleotide (Fig. 8B, lanes 3 and 4), and the 3'-CAGGTG E-box and the μE5 oligonucleotides...
Fig. 5. Deletion analysis of the HS I silencer. 5' and 3' deletions of the −8025/−7059 DNA fragment that contained HS I were linked to the −96/+598 human GATA-3 promoter and cloned upstream from the CAT reporter gene. The different constructs were stably transfected into T-cell (Jurkat; □), non-hematopoietic (HeLa; □), and erythrocytic (K562; □) cell lines, and the CAT activities were quantified using a PhosphorImager. The values represent the average of three independent pools of transfected cells, and 1 represents the CAT activity obtained with the pBL-CAT-3 vector. The bent arrows indicate the human GATA-3 transcriptional initiation site. The white boxes represent the first exon of the human GATA-3 gene.

GATA-3 Gene Regulation in T-cells

To demonstrate that ZEB was involved in human GATA-3 gene silencer. The −7828/−7121 DNA fragment containing the human GATA-3 gene silencer was sequenced on both strands. The potential DNA-binding sites present in the 5' and 3' elements defined by the deletion analysis of the HS I silencer are indicated in boldface letters. The nucleotide sequence has been submitted to the GenBank™/EBI Data Bank with accession number AJ131811.

Fig. 6. Sequence of the human GATA-3 gene silencer. The −7828/−7121 DNA fragment containing the human GATA-3 gene silencer was sequenced on both strands. The potential DNA-binding sites present in the 5' and 3' elements defined by the deletion analysis of the HS I silencer are indicated in boldface letters. The nucleotide sequence has been submitted to the GenBank™/EBI Data Bank with accession number AJ131811.

GATA-3 Gene Regulation in T-cells
gene repression, we cotransfected a ZEB expression vector together with the human GATA-3 promoter linked to the −7828/−7121 DNA fragment, and the resulting constructs were linked to the −96/+598 human GATA-3 promoter and cloned upstream from the CAT reporter gene. The different constructs were stably transfected into T-cell (Jurkat; □), non-hematopoietic (HeLa; □), and erythrocytic (K562; □) cell lines, and the CAT activities were quantified using a PhosphorImager. The values represent the average of three independent pools of transfected cells, and 1 represents the CAT activity obtained with the pBL-CAT-3 vector. The bent arrows indicate the human GATA-3 transcriptional initiation site. The white boxes represent the first exon of the human GATA-3 gene. B, a point mutation of the 5′-CAGGTG E-box was performed on the −7828/−7121 DNA fragment, and the mutated fragment was analyzed as described for A.

We then performed several point mutations of the 3′-CAGGTTG E-box, which can bind ZEB, E2A/HEB, and USF proteins. We transformed this 3′-E-box to a MEF1m sequence, i.e. a sequence that can weakly bind ZEB and neither E2A/HEB nor USF (Fig. 8B, lane 7) (26); to the sequence AGTTCAGGTGTGTT located at −361 in the human α4-integrin promoter and shown to bind only ZEB and USF proteins (30, 31); or to a xE2 sequence known to bind only ZEB and E2A/HEB proteins (32). Stable transfections of the resulting constructs in HeLa and Jurkat T-cells showed that the mutated xE2 sequence had the same effect as the wild type 3′-E-box, whereas the MEF1m or −361 sequence induced a repression of the human GATA-3 promoter in Jurkat T-cells (Fig. 9B). These results indicate that the E2A/HEB heterodimer, but not the USF proteins, can relieve the repression mediated by ZEB on the 3′-E-box.

DISCUSSION

Among the GATA transcription factors, GATA-3 displays a peculiar expression, as it is expressed in many different tissues (i.e. central and peripheral nervous systems and embryonic liver, kidney, and adrenal medulla) during development and
FIG. 8. A, cell-type specificity of the 3'-CAGGTG E-box complexes. HeLa (lanes 1 and 2) and Jurkat (lanes 3 and 4) nuclear extracts were tested for binding activity with the end-labeled 3'-CAGGTG E-box oligonucleotide probe in an electrophoretic mobility shift assay. The three specific complexes (C1, C2, and C3) are indicated by arrows. B, mapping of the 3'-CAGGTG protein-DNA complexes. μE5 (lane 3), 3'-CAGGTG E-box (lane 4), and MEF1m (lane 7) oligonucleotides were used as probes in an electrophoretic mobility shift assay with Jurkat nuclear extract. Cold competition of μE5 binding with μE5 (lane 1) or the 3'-E-box (lane 2), of 3'-E-box binding with the 3'-E-box (lane 5) or μE5 (lane 6), and of MEF1m binding with the 3'-E-box (lane 8) is shown. The specific C1, C2, and C3 complexes are indicated. C, anti-ZEB antibodies induce supershift of the C1 complex. The 3'-CAGGTG E-box oligonucleotide was end-labeled and incubated with a nuclear extract from HeLa cells in the presence of a preimmune (lane 1) or an immune (lane 2) serum against ZEB. Similar results were obtained with a nuclear extract from Jurkat cells, but are not shown as the C3 complex migrated at the same position as the anti-ZEB supershift complex. The C1 and C2 complexes are indicated by arrows. D, supershift of the C3 complex with an anti-E12/E47 or anti-HEB antibody. The end-labeled 3'-CAGGTG E-box oligonucleotide was incubated with a nuclear extract from Jurkat cells in the presence of a preimmune serum (lane 1) or antibodies against E12/E47 (lane 2) or HEB (lane 3). The C1, C2, and C3 complexes are indicated by arrows. E, supershift of the C2 complex with anti-USF1 or anti-USF2 antibodies. The 3'-CAGGTG E-box oligonucleotide was end-labeled and incubated with a nuclear extract from Jurkat cells in the presence of a preimmune (lane 1) or immune (lane 4) serum against USF1 or USF2 (lane 3) or a mixture of the two immune sera (lane 2). The preimmune serum used in this experiment drastically decreased the C3 complex. The specific C1 and C2 complexes are indicated by arrows.
only in the placenta, the central nervous system, very immature hematopoietic cells, and T-cells in the adult. To define regulatory sequences that control GATA-3 gene expression in T-cells, we first investigated the DNase-I hypersensitivity of the human GATA-3 gene as DNase-I hypersensitivity has been associated with a wide range of cis-regulatory sequences and is usually indicative of protein-DNA interactions. Although no T-cell-specific HS site was found 5’ from the human GATA-3 gene initiation site, three T-cell-specific HS sites were discovered in the 3’-direction. We assayed these different T-cell-specific HS sites, linked to the endogenous or to a heterologous promoter, in both transient and stable transfections, but were unable to show any function of these HS sites in transcriptional regulation. These results indicate a possible requirement for other regulatory elements or that the assays we used could not detect the function of these DNase-I HS sites.

We first determined the cis-acting sequences, located near the transcriptional initiation site and involved in the human GATA-3 gene activation. We did not find any sequence involved in the T-cell-specific activity of the human GATA-3 gene, but mapped a sequence, located in the first intron between +475 and +588, required for high transcriptional activity. This sequence did not display classical enhancer activity, as it functioned, in both sense and antisense orientations, only when located 3′ from the GATA-3 minimal promoter. Similar results have been obtained using the mouse GATA-3 promoter (14), and comparison of the mouse and human sequences located at the end of the first intron showed little homology except at their 3′-ends, where the sequence 5′-CAGGTCTC(C/T)-3′ lies (in the human and murine introns) one base 5′ from the 3′-splicing sequence.

We then studied the effects of the 5′-HS sites on the transcriptional activity of the human GATA-3 promoter and found that a DNA fragment, located between −8025 and −5900, could confer T-cell specificity to the human GATA-3 promoter. This T-cell specificity is mainly mediated by a strong silencer active in non-T-cells and located between −7828 and −7121. Previous studies have shown an extinction of the GATA-3 gene...
GATA-3 Gene Regulation in T-cells

We have previously shown that a cosmid that contains the human GATA-3 transcription unit extended by 4 kb of 3′-flanking sequence and 3 kb of 5′-flanking sequence is sufficient for directing T-cell expression (18). This cosmid does not contain the silencer described in this paper, and thus, the human GATA-3 gene seems to be under the control of discrete regulatory elements that can enhance and/or silence the transcriptional activity of a minimal promoter. Furthermore, a recent publication has characterized an enhancer sequence that regulates mouse GATA-3 gene expression in the brachial arch (44). This enhancer, located between nucleotides –2832 and –2642, does not confer any T-cell specificity, and thus, the GATA-3 gene seems to be regulated by discrete regulatory elements required for its complex expression pattern. Such a modular cis-regulatory organization has been described for many genes encoding transcription factors in Drosophila (15) and shows the actual complexity of cis-regulatory elements that regulate these genes.

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REFERENCES

1. Shivdasani, R. A., and Orkin, S. H. (1996) Blood 87, 4025–4039
2. Orkin, S. H. (1992) Blood 80, 575–581
3. Penney, L., Simon, M. C., Robertson, E., Klein, W. H., Tsai, S.-F., D’Agati, V., Orkin, S. H., and Costantini, F. (1991) Nature 350, 257–260
4. Tsai, F.-Y., Keller, G., Kuo, F. C., Weiss, M. J., Chen, J., Rosenblatt, M., Alt, F., and Orkin, S. (1994) Nature 371, 221–227
5. Pandolfi, P. P., Roth, M. E., Karsa, A., Leonard, M. W., Dzierzak, E., Grosveld, F. G., Engel, J. D., and Lindenbaum, M. H. (1995) Nat. Genet. 11, 40–44
6. Ting, C.-N., Olson, M. C., Barton, K. P., and Leiden, J. M. (1996) Nature 384, 474–478
7. Tsai, S.-F., Martin, D. I. K., Zon, L. I., D’Arenda, A. D., Wang, G. G., and Orkin, S. H. (1989) Nature 339, 446–451
8. Martin, D. I. K., Zon, L. I., Mutter, G., and Orkin, S. H. (1990) Nature 344, 444–446
9. Romeo, P.-H., Prandini, M.-H., Joulion, V., Mignotte, V., Prentaz, M., Vainchenker, W., Marguerie, G., and Uzan, G. (1990) Nature 344, 447–451
10. Yamamoto, M., Ko, L. J., Leonard, M. W., Beug, H., Orkin, S. H., and Engel, J. D. (1990) Genes Dev. 4, 1560–1562
11. Mouthon, M.-A., Bernard, O., Mitjavila, M.-T., Romeo, P.-H., Vainchenker, W., and Mathieu-Mahul, D. (1993) Blood 81, 647–655
12. Biassoni, R., Verdiani, S., Cambiaggi, A., Romeo, P.-H., Ferrini, S., and Moretta, L. (1993) Eur. J. Immunol. 23, 1083–1087
13. Joulion, V., Bories, D., Eloet, J.-F., Labastie, M.-N., Chrétien, S., Mattei, M.-G., and Romeo, P.-H. (1991) EMBO J. 10, 1809–1816
14. George, K. M., Leonard, M. W., Roth, M. E., Lieuw, K., Kuusius, D., Grosveld, F., and Engel, J. D. (1994) Development (Camb.) 120, 2673–2686
15. Arnone, M. I., and Davidson, E. H. (1997) Development (Camb.) 124, 1851–1864
16. Murrell, A. M., and Green, A. R. (1995) Oncogene 16, 631–639
17. Sawada, S., Scarborough, J. D., Killeen, N., and Littman, D. R. (1994) Cell 77, 917–929
18. Labastie, M.-N., Bories, D., Chabret, C., Grégoire, J.-M., Chrétien, S., and Romeo, P.-H. (1994) Genomics 21, 1–6
19. Ishihara, H., Engel, J. D., and Yamamoto, M. (1995) J. Biochem. (Tokyo) 99, 499–508
20. Luckow, B., and Schultz, G. (1987) Nucleic Acids Res. 15, 5490
21. Ellis, L., Clauer, E., Morgan, D. O., Edery, M., Roth, B. A., and Burton, W. J. (1986) Cell 45, 721–732
22. Rosenthal, N. (1987) Methods Enzymol. 152, 704–734
23. Ausubel, F. M., Brent, R., Kingdom, E. R., Moore, D. M., Seidman, J. G., Smith, J. A., and Struhl, K. (1989) Current Protocols in Molecular Biology, Vol. 1, pp. 9.6.13–9.6.14, John Wiley & Sons, Inc., New York
24. Schreiber, E., Matthias, P., Muller, M., and Schaﬀner, W. (1989) Nucleic Acids Res. 17, 6419
25. Hsu, H.-L., Huang, L., Tsou Tean, J., Funk, W., Wright, W. E., Hu, J.-S., Kingdom, E. R., and Baer, R. (1994) Mol. Cell. Biol. 14, 1256–1265
26. Genetta, T., Ruzinsky, D., and Kadesch, T. (1994) Mol. Cell. Biol. 14, 6153–6163
27. Bonifacino, J. S., Silber, T., Gropveld, S., and Sippel, E. E. (1990) EMBO J. 9, 2843–2849
28. Grosveld, F., Blom von Assendelft, G., Greaves, D. R., and Kollia, G. (1987) EMBO J. 6, 975–988
29. Meng, A., Tang, H., Ong, B. A., Farrell, M. J., and Lin, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6267–6272
30. Postigo, A. A., Sheppard, A. M., Mucenski, M., and Dean, D. C. (1997) EMBO J. 16, 3924–3934
31. Postigo, A. A., and Dean, D. C. (1997) EMBO J. 16, 3935–3943
32. Sekido, R., Murai, K., Funahashi, J. I., Kamachi, Y., Fujisawa-Sehara, A., Nabelshima, Y. I., and Kendoh, H. (1994) Mol. Cell. Biol. 14, 5692–5709
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33. Zheng, W.-P., and Flavell, R. A. (1997) Cell 89, 587–596
34. Saksela, K., and Baltimore, D. (1993) Mol. Cell. Biol. 13, 3698–3705
35. Goraya, T. Y., Kessler, S. P., Kumar, R. S., Douglas, J., and Sen, G. C. (1994) Nucleic Acids Res. 22, 1194–1201
36. Winoto, A., and Baltimore, D. (1989) Cell 59, 649–655
37. Liu, Z. Y., Chin, K., and Noguchi, C. T. (1994) Dev. Biol. 166, 159–169
38. Vitale, M., Di Marzo, R., Calzolari, R., Acuto, S., O’Neill, D., Bank, A., and Maggio, A. (1994) Biochem. Biophys. Res. Commun. 204, 413–418
39. Markowitz, A. J., Wu, G. D., Bafer, A., Cui, Z., Chen, C. L., and Traber, P. G. (1995) Am. J. Physiol. 269, G925–G939
40. Cooney, A. J., Tsai, S. Y., O’Malley, B. W., and Tsai, M.-J. (1992) Mol. Cell. Biol. 12, 4153–4163
41. Galvin, K. M., and Shi, Y. (1997) Mol. Cell. Biol. 17, 3723–3732
42. Zhuang, Y., Cheng, P., and Weintraub, H. (1996) Mol. Cell. Biol. 16, 2898–2905
43. Sawada, S., and Littman, D. R. (1993) Mol. Cell. Biol. 13, 5620–5628
44. Lieuw, K. H., Li, G.-l., Zhou, Y., Gravveld, F., and Engel, J. D. (1997) Dev. Biol. 188, 1–16