An NK and T Cell Enhancer Lies 280 Kilobase Pairs 3' to the *Gata3* Structural Gene[∇]

Sakie Hosoya-Ohmura,¹ Yu-Hsuan Lin,¹ Mary Herrmann,¹ Takashi Kuroha,¹ Arvind Rao,¹ Takashi Moriguchi,^{1,2} Kim-Chew Lim,¹ Tomonori Hosoya,¹ and James Douglas Engel^{1*}

Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Michigan 48103,¹ and Department of Medical Biochemistry, Tohoku University, Graduate School of Medicine, Aoba-ku, Sendai, Miyagi 980-8575, Japan²

Received 17 January 2011/Returned for modification 9 February 2011/Accepted 18 February 2011

Transcription factor GATA-3 is vital for multiple stages of T cell and natural killer (NK) cell development, and yet the factors that directly regulate *Gata3* transcription during hematopoiesis are only marginally defined. Here, we show that neither of the *Gata3* promoters, previously implicated in its tissue-specific regulation, is alone capable of directing *Gata3* transcription in T lymphocytes. In contrast, by surveying large swaths of DNA surrounding the *Gata3* locus, we located a *cis* element that can recapitulate aspects of the *Gata3*-dependent T cell regulatory program *in vivo*. This element, located 280 kbp 3' to the structural gene, directs both T cell- and NK cell-specific transcription *in vivo* but harbors no other tissue activity. This novel, distant element regulates multiple major developmental stages that require GATA-3 activity.

Maturation of T lineage lymphocytes is one of the most clearly defined pathways in all of developmental biology. Immature hematopoietic cells from the bone marrow migrate through the bloodstream to initially populate the thymus. The earliest detectable thymic progenitor (early T lineage progenitors [ETP]) cells differentiate uniquely in the thymic microenvironment through several early stages in which neither the CD4 nor CD8 coreceptors are expressed (double-negative [DN] cells, stages 2 to 4) and thence into cells which express both CD4 and CD8 (double-positive [DP] cells) and finally generate either CD4 single-positive (CD4 SP; CD4⁺ CD8⁻) cells or CD8 single-positive (CD8 SP; CD4⁻ CD8⁺) cells. CD4⁺ thymocytes have the potential to differentiate into helper (Th) or regulatory T cells, while CD8 SP cells are programmed to fulfill cytotoxic functions. These mature single-positive thymocytes exit the thymus to execute their defined effector functions after activation in the periphery.

The zinc-finger transcription factor GATA-3 (23, 49) is expressed throughout T cell development (20), peaking in abundance in CD4 SP and Th2 cells (11, 17, 18, 38, 44, 52, 53). GATA-3 function has been shown to be vital for the generation of ETP (19), DN4, and CD4 SP cells (37) and for the differentiation and function of Th2 cells (36, 54). While its expression is critical for normal T cell development, enforced ectopic expression of GATA-3 can have catastrophic consequences (3, 7, 33, 34, 40, 41), such as causing T cell lymphoma in transgenic mice (34, 45) and converting DP cells into a premalignant state (45). Additionally, GATA-3 plays a role in the aberrant survival of T lymphoma cells in E2A mutant mice (48). These results, taken to-

gether, suggest that both the timing and abundance of GATA-3 must be exquisitely regulated for proper development of the T cell lineage.

Ours and many other laboratories have sought to define how this key T lymphocyte regulatory protein is itself so precisely modulated during T cell development, but prior studies have failed to conclusively identify *Gata3* transcriptional *cis* elements that are capable of conferring appropriate regulatory properties to this gene *in vivo*. In exploring the transcriptional networks that lead to proper T cell differentiation, potential *trans* upstream regulators of *Gata3* have been proposed (1, 12, 31, 50, 51). However, while *Gata3* proximal promoter sequences are capable of activating its transcription in transfection experiments (14), we report here that neither of the *Gata3* promoters (4) is capable of conferring such activity *in vivo*. Since previous studies have not demonstrated a functional requirement for the direct association of any of the proposed epistatic *Gata3* regulators (Notch/CSL, c-Myb, T cell factor 1 [TCF-1], and Dec2) with their cognate binding sites in the *Gata3* promoters through site mutagenesis followed by *in vivo* activity tests, the experiments described here clearly demonstrate that those sequences are insufficient for thymic T cell-specific expression.

We and many others have shown that GATA-3 plays critical roles in quite diverse developmental events (5, 15, 21, 24, 25, 30, 32, 43) and that *Gata3* expression in those tissues and organs is usually dictated by individual tissue-specific enhancers (14, 16, 26, 27, 29). Here, we report the identification of a *cis* element that regulates *Gata3* expression during multiple stages of T cell development and that is located far 3' to the *Gata3* gene. This element induces the transcription of a reporter gene *in vivo* in thymic ETP, natural killer (NK), $\gamma\delta$ T, CD4 SP, and peripheral CD4⁺ stages and thus its expression pattern reflects that of endogenous *Gata3*. While additional *cis* elements may be required to fully support appropriate expression of *Gata3* during T cell development, this distant element appears to confer activity at several of the major developmen-

* Corresponding author. Mailing address: Department of Cell and Developmental Biology, 3071 BSRB, 109 Zina Pitcher Place, University of Michigan Medical School, Ann Arbor, MI 48103. Phone: (734) 904-0944. Fax: (734) 615-8500. E-mail: engel@umich.edu.

[∇] Published ahead of print on 7 March 2011.

tal transitions that are required for *Gata3* T cell-specific transcriptional control *in vivo*.

MATERIALS AND METHODS

Mice. Transgenic mice were generated using standard techniques in the University of Michigan Transgenic Animal Model Core or using our own instruments. Bacterial artificial chromosome (BAC) or plasmid DNAs were microinjected into (C57BL/6J × SJL)F2 fertilized oocytes. Transgenic lines were established by crossing onto a CD1 background. GATA-3-enhanced green fluorescent protein (eGFP) fusion cDNA knock-in mice (*Gata3^{gfp}*) were generated previously (19) (T. Moriguchi et al., unpublished data). *Gata3^{2/+}* mice (17) and *Tg^{B125.LacZ}* mice (the genome sequence-revised endpoints are −451 to +211 kb, with respect to the translation start site) were described previously (16, 26, 27). All animal experiments were approved by the University Committee on Use and Care of Animals of the University of Michigan and were performed according to their guidelines (IACUC approval no. 8611).

BAC recombineering. The RPCI-23 C57BL/6J mouse BACs used in this study are described by the following endpoints (±0.5 kbp) relative to the *Gata3* translational start site: 43G17, +49/+294; 193E6, +128/+330; 263A8, +269/+473. Modification of BAC clones was performed as previously described (22, 28).

eGFP reporter BAC recombinants. The pGATA-3-eGFP fusion cDNA plasmid containing the genomic *Ib* promoter (unpublished data) was digested with NcoI and self-ligated to remove only the *Gata3* cDNA; this is referred to as the pG3-*Ib*-eGFP plasmid. pG3-*Ib*-eGFP was digested with EcoRI and NotI to excise the fragment containing the *Ib* promoter (sequences corresponding to bp −1314 to +1, with respect to the translational initiation site) and eGFP cassette. A pG3 BAC-targeting vector (unpublished data), which contains an Frt-Neo-Frt selection cassette, the *Ib*-LacZ reporter, and two homology arms that were identical to two segments of the *SacBII* gene in the pBACe3.6 vector backbone, was digested with EcoRI and NotI to remove the *Ib*-LacZ cassette. The EcoRI-NotI fragment of pG3-*Ib*-eGFP and the EcoRI-NotI fragment of the pG3 BAC-targeting vector were ligated to generate the *Ib*-eGFP BAC-targeting plasmid. The resultant plasmid was digested, purified, and used for BAC homologous recombination. The recombinant BAC clones were verified by restriction enzyme digest pattern and Southern blotting (data not shown).

Deletion of *TCE-7.1* from BAC 43G17. Two homology arms that were located immediately adjacent to either end of *TCE-7.1* were amplified by PCR and then subcloned into the pFrtNeo plasmid, which contains the Frt-Neo-Frt cassette (22). BAC homologous recombination was performed using the purified targeting fragment as described previously (22). The resultant recombinant BAC (43G17Δ*TCE-7.1*) was verified by restriction enzyme digestion and Southern blotting (data not shown).

Construction of an eGFP reporter plasmid containing *TCE-7.1*. To prepare the *Ib*-eGFP reporter plasmid, the Neo cassette was removed from the *Ib*-eGFP BAC-targeting plasmid. BAC 43G17 DNA was digested with SalI and KpnI. The 7.1-kbp SalI-KpnI fragment (*TCE-7.1* fragment) was gel purified and cloned into the SalI/KpnI sites of pGEM-4Z. The *TCE-7.1* fragment was verified by restriction digestion pattern and sequencing. To generate the 7.1-*Ib*-eGFP reporter plasmid, the SalI-KpnI *TCE-7.1* fragment from the pGEM-4Z 7.1-kbp plasmid, the PacI-SalI fragment of the *Ib*-eGFP plasmid, and the PacI-KpnI fragment of pNEB193 were ligated together. The resultant 7.1-*Ib*-eGFP plasmid was digested with PmeI and KpnI and used for microinjection. To generate *Gata3-Ib* promoter-only transgenic mice, the *Ib*-eGFP plasmid was digested with PacI and PmeI and used for microinjection.

Flow cytometry. Single-cell suspensions of thymocytes, bone marrow, splenocytes, or peripheral blood were incubated with Fc Block (BD Biosciences). Splenocytes and peripheral blood were hemolyzed using NH₄Cl before incubation with Fc Block. The following antibodies (either from eBioscience or from BD Biosciences) were then applied: phycoerythrin-cyanine 7-conjugated (PE-Cy7) anti-CD4 (RM4-5), PE-anti-CD4 (H129.19), allophycocyanin (APC)-anti-CD8a (53-6.7), biotin-anti-CD8a (53-6.7), PE-anti-CD44 (IM7), peridinin chlorophyll protein-Cy5.5 (PerCP-Cy5.5)-anti-CD62L (MEL-14), PE-Cy7-anti-CD25 (PC61.5), APC-anti-c-Kit (2B8), PE-anti-CD3e (145-2C11), PE-Cy7-anti-CD3e (145-2C11), biotin-anti-CD3e (145-2C11), APC-anti-γδTCR (T cell receptor) (GL3), biotin-anti-γδTCR (GL3), PE-Cy7-anti-CD19 (1D3), biotin-anti-CD19 (1D3), APC-anti-CD49b (DX5), APC-anti-B220 (RA3-6B2), biotin-anti-B220 (RA3-6B2), APC-eFluor780-anti-Mac1 (M1/70), biotin-anti-Mac1 (M1/70), eFluor450-anti-Gr1 (RB6-8C5), biotin-anti-Gr1 (RB6-8C5), APC-anti-TER119 (TER-119), biotin-anti-TER119 (TER-119), PE-anti-CD71 (R17217), PerCP-Cy5.5-anti-TCRβ (H57-597), biotin-anti-TCRβ (H57-597), PE-anti-CD69 (H1.2F3), biotin-anti-NK1.1 (PK136), biotin-anti-CD11c

(N418), PE-Cy7-anti-gamma interferon (anti-IFN-γ) (XMG1.2), APC-anti-interleukin 4 (anti-IL-4) (11B11), PE-Cy7-anti-Sca1 (D7), streptavidin eFluor450. Immature T cells were analyzed as previously described (19). The FluorReporter LacZ flow cytometry kit (Molecular Probes) was used to analyze LacZ expression according to the manufacturer's protocol. Cells were analyzed on either FACSCanto II (BD Biosciences) or FACSCalibur (BD Biosciences). Dead cells were excluded by DAPI (4',6-diamidino-2-phenylindole) or propidium iodide. Acquired data were analyzed using either Weasel (WEHI Biotechnology Centre), FlowJo (Tree Star, Inc.), FACSDiva, or Cell Quest (BD Biosciences) software. The mean fluorescence intensity (MFI) of eGFP was normalized using the LinearFlow green flow cytometry intensity calibration kit (Molecular Probes) in most experiments. These calibration beads were excited by 488 nm, and fluorescence measurements were performed in the same manner as eGFP measurements in every experiment. A standard curve was generated based on acquired calibration bead data, and eGFP MFI of each sample was normalized using the standard curve.

In vitro CD4⁺ T cell differentiation assay. CD4⁺ CD25[−] splenocytes were purified using the Dynal mouse CD4-negative isolation kit (Invitrogen) in combination with affinity-purified anti-mouse CD25 antibody (PC61.5; eBioscience) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 4 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-mercaptoethanol, 25 mM HEPES, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate. Isolated CD4⁺ cells were stimulated with plate-bound anti-CD3e antibody (10 μg/ml; 145-2C11; BD Biosciences) and anti-CD28 antibody (10 μg/ml; 37.51; BD Biosciences). For nonpolarizing conditions, 10 ng/ml recombinant human IL-2 (PeproTech) was added. For Th1-polarizing condition, 10 μg/ml anti-IL-4 antibody (11B11; BD Biosciences), 10 ng/ml IL-2, and 5 ng/ml recombinant mouse IL-12 (PeproTech) were added. For Th2-polarizing condition, 10 μg/ml anti-IFN-γ antibody (XMG1.2; BD Biosciences), 10 μg/ml anti-IL-12 antibody (C17.8; BD Biosciences), 10 ng/ml IL-2, and 10 ng/ml recombinant mouse IL-4 (PeproTech) were added. On day 4 of culture, cells were diluted. On day 6, cells were restimulated with plate-bound anti-CD3e and anti-CD28 antibodies for 6 h. During the last 2 h, 10 μg/ml brefeldin A (Sigma-Aldrich) was added. Half of the cells were analyzed using flow cytometry to evaluate eGFP expression, while the other half were fixed with 4% paraformaldehyde, permeabilized with Perm/Wash buffer (BD Biosciences), and used for intracellular staining to confirm differentiation by detecting IFN-γ and IL-4 (data not shown).

In vivo and ex vivo imaging. Postnatal day 4 mice (anesthetized with isoflurane vapor) or fresh organs from adult mice were analyzed using an IVIS spectrum (Caliper Life Sciences). The excitation and emission wavelengths used in this study were 465 nm and 520 nm, respectively. Acquired data were analyzed using Living Image 4.0 software.

Bioinformatics. Comparisons of mouse genomic sequences with human, dog, and rat were performed using VISTA (<http://genome.lbl.gov/vista/index.shtml>). The information describing the position of DNase I hypersensitive sites (DHSs) in human CD4⁺ T cells were obtained from the UCSC genome browser (6, 8–10, 47).

ESTs. The information regarding the two expressed sequence tags (ESTs) that are located within 300 kbp of *TCE-7.1* is as follows: *AK080422*, *Mus musculus* 7-day neonate cerebellum cDNA, RIKEN full-length enriched library, clone ID A730010B06 (http://genome.ucsc.edu/cgi-bin/hgGene?hgg_gene=uc008ihc.1&hgg_prot=&hgg_chrom=chr2&hgg_start=9512845&hgg_end=9519470&hgg_type=knownGene&db=mm9&hgsid=186400201); and *AK035738*, *Mus musculus* adult male urinary bladder cDNA, RIKEN full-length enriched library, clone ID 9530097M04 (http://genome.ucsc.edu/cgi-bin/hgGene?hgg_gene=uc008ihb.1&hgg_prot=&hgg_chrom=chr2&hgg_start=9274116&hgg_end=9369303&hgg_type=knownGene&db=mm9&hgsid=186400201).

RESULTS

Neither the *Gata3-1a* nor *-1b* promoter confers T cell autonomous expression *in vivo*. Given our previous report that sequences in the *Gata3-Ib* (gene-proximal) promoter exerted differential T cell activity in transfection experiments (14), we first asked whether the same promoter was capable of directing T cell transcription *in vivo*. Transgenic mice were generated in which an eGFP reporter cassette was directed by *Ib* promoter sequences (*Ib*-eGFP), and expression in T cells was monitored by flow cytometry. Surprisingly, the reporter gene failed to be

FIG. 1. Neither the *Gata3-1b* promoter alone nor a 662-kbp *Gata3/LacZ* YAC containing both *1a* and *1b* promoters recapitulates GATA-3 activity in thymocytes. (A) eGFP expression in CD4 SP thymocytes from *Gata3^{g/+}* mice (left, gray shaded histogram) or *Tg^{1b.eGFP}* mice (right, gray shaded histogram). The black-line (open) histograms indicate eGFP fluorescence in wild-type thymocytes. Data represent at least three mice of each genotype. (B) LacZ expression in thymocytes stained with anti-CD4 and anti-CD8 antibodies was examined by flow cytometry. Each population was gated as depicted. The gray shaded histograms indicate fluorescein di- β -D-galactopyranoside fluorescence (13) resulting from hydrolysis due to β -galactosidase expression in either *Gata3-lacZ* knock-in (*Gata3^{z/+}*) (17) or B125-*lacZ* YAC transgenic (27) mice, while the black-line (open) histograms indicate expression in wild-type mice. These individual data are representative of results from at least three mice of each genotype.

expressed in T cells of adult transgenic (*Tg^{1b.eGFP}*) mice (Fig. 1A). In contrast, mice expressing a germ line eGFP-GATA-3 fusion protein (*Gata3^{g/+}*) (Fig. 1A) or a germ line *lacZ* insertion at the *Gata3* initiation codon (*Gata3^{z/+}*) (Fig. 1B) both robustly express the reporters in T cells (17, 19). These data demonstrate that the *Gata3-1b* promoter is insufficient to confer T cell-specific transcription *in vivo* and, therefore, that an additional *cis* element(s) is required.

A potent T cell element located far 3' to *Gata3*. We previously generated B125 yeast artificial chromosome (YAC) *LacZ* reporter transgenic mice harboring 662 kbp of genomic DNA containing the 33-kbp *Gata3* structural gene as well as vast swaths of adjacent 5' (451-kbp) and 3' (211-kbp) genomic noncoding sequences (Fig. 1B, *Tg^{B125.LacZ}*) (26, 27). We compared β -galactosidase expression in the thymocytes of *Tg^{B125.LacZ}* mice and *LacZ* germ line knock-in (*Gata3^{z/+}*) animals. Surprisingly, *LacZ* expression was not observed in adult *Tg^{B125.LacZ}* thymocytes (Fig. 1B). In contrast, the *Gata3^z* germ line knock-in allele was strongly expressed, most robustly in the CD4 SP population that also abundantly expresses endogenous GATA-3 in thymocytes (Fig. 1B). These data demonstrate that even 662 kbp of contiguous genomic sequence, including the gene and both *Gata3* promoters, was insufficient to direct

Gata3 T cell transcription *in vivo* and that the *cis* elements required for T lineage specification must be located beyond the boundaries of that YAC.

Next, we began to examine sequences lying even further away using the coupled BAC/transgenic (BAC-trap) assay we developed and exploited previously to identify several distant *Gata2* urogenital enhancers (22). Three BACs that overlapped and extended 3' to the B125 YAC (Fig. 2A) were modified by recombineering (28) to insert an eGFP reporter gene directed by the *Gata3-1b* (gene-proximal) promoter (*1b.eGFP*) into each BAC vector backbone (Fig. 2B). The *1b* promoter was examined (instead of the more distal *1a* promoter) in these studies since more than 98% of peripheral T cell transcripts initiate from exon *1b* (51). The three recombineered BACs were used to generate founder transgenic animals, and robust eGFP expression was detected in thymocytes from multiple transgenic animals (Fig. 2C and data not shown) using all three BACs. Although eGFP expression initially appeared to differ between the different BAC clones, after recovery of multiple founders bearing each clone (and after subsequent analysis of multiple BAC transgenic lines), we concluded that the heterogeneity was due to mosaic expression of the transgenes in the founder transgenic mice. Based on the T cell-directed eGFP

FIG. 2. A candidate T lymphocyte enhancer element is located far 3' to the *Gata3* gene. (A) The *Gata3* gene and adjacent genes on mouse chromosome 2. The relative genomic positions of the BAC and YAC clones examined in this study are depicted graphically. (B) Schematic diagram of the targeting cassette used to generate modified BACs. H1 and H2, homology arms; Neo, neomycin resistance gene; *G3P*, *Gata3* promoter; SacBII, SacBII gene present in the vector backbone of the RPCI-23 mouse BAC library; CmR, chloramphenicol acetyltransferase gene C, founder screening of BAC-trap Tg embryos. eGFP expression in total thymocytes recovered from E18.5 F₀ Tg embryos was analyzed by flow cytometry. The results of two independent F₀ Tg embryos for each BAC clone are shown; in each case, a fraction of the thymocytes expressed eGFP, except from wild-type embryos.

responses observed in this founder screen, we immediately focused on the region of overlap between the three BAC clones.

Preliminary bioinformatic analysis revealed (Fig. 3A and B) that multiple species-conserved noncoding sequence (CNS) elements lie within the overlap (approximately 25 kbp) between the RPCI-23 library BACs 43G17 and 263A8. We further informed the analysis by aligning DHS data from primary human CD4⁺ T cells (6, 8–10, 47) (Fig. 3C); several of the CNS

were close to, or overlapped, DHS sites (Fig. 3). Given the close relationship between conserved sequence elements and DHS sequences with transcriptional control, we hypothesized that the most highly conserved CNS overlapping the DHS (shown in Fig. 3B) might serve as a *cis* regulatory element that controls T cell-specific *Gata3* transcription.

A 7.1-kbp genomic fragment directs *Gata3* activity at multiple T cell stages. Within the 25-kbp overlapping BAC inter-

FIG. 3. Mapping conserved noncoding sequences (CNS) and DNase I hypersensitive sites (DHS) in the overlap between two BAC clones. (A) The region of overlap (approximately 25 kbp) between BACs 43G17 and 263A8 is depicted. (B) Genomic sequences within the overlap (mouse chromosome 2, 9,530,005 to 9,504,884) were compared with the human, dog, and rat genomes, respectively. CNSs are colored pink. (C) The DHS homologues corresponding to human CD4⁺ T cells are depicted as gray rectangles. Open arrows in panel B indicate CNSs that were predicted to be potential regulatory elements (i.e., high ESPERR scores).

val, we initially focused on a 7.1-kbp SalI/KpnI restriction fragment that contained multiple CNS elements and that also had high predicted regulatory sequence potential (evolutionary and sequence pattern extraction through reduced representations [ESPERR]) scores (42) (data not shown); additionally, this fragment encompassed most of the DHS (Fig. 3), and therefore we assigned to it the preliminary designation *TCE-7.1* (7.1-kbp T cell element). First, we asked whether or not this fragment contained T cell enhancer activity by deleting the corresponding region ($\Delta 7.1$) via recombineering from the BAC 43G17 clone into which a *Ib.eGFP* reporter cassette had already been inserted. We chose to examine BAC 43G17 instead of the two others simply because we first observed transcription of eGFP reporter gene in thymocytes of Tg^{43G17} mice (data not shown). The deletion BAC was then used to generate transgenic mice (Tg^{43G17 Δ 7.1}). eGFP fluorescence was conspicuously absent in peripheral CD4⁺ cells in all founder Tg^{43G17 Δ 7.1} mice (0/14 transgenic mice expressed eGFP fluorescence) compared to that in the cells of the parental Tg^{43G17} mice (Table 1), indicating that *TCE-7.1* is necessary for direc-

tion of reporter gene transcription in T cells. To ask if that same fragment alone was sufficient to enhance T cell transcription, it was linked to the same *Ib.eGFP* reporter cassette that was used in the BAC vector modification recombineering experiments; this reporter was then used to generate founder transgenic mice (Tg^{7.1-*Ib.eGFP*}). We found that eGFP in peripheral CD4⁺ T cells increased in all founder mice (6/6) bearing *TCE-7.1* linked to the promoter compared to in transgenic mice bearing the *Ib* promoter alone (Table 1).

We next established transgenic lines bearing each of these reporters and assayed the lines for when (during T cell development) and where (in which organs) the transgenes were expressed. We initially analyzed thymocytes from multiple established transgenic lines: six lines of Tg^{7.1-*Ib.eGFP*}, four lines of Tg^{*Ib.eGFP*}, five lines of Tg^{43G17}, and three lines of Tg^{43G17 Δ 7.1}. Thymocytes were electrically gated into DN1 to DN4, DP, CD4SP, and CD8SP stages using anti-CD4, CD8, CD25, and CD44 antibodies; we found that eGFP was expressed in those cells in all lines of both Tg^{43G17} and Tg^{7.1-*Ib.eGFP*} mice. In contrast, eGFP expression was not observed in the absence of *TCE-7.1* (data not shown). Based on those pilot experiments, we chose two lines of each construct-derived transgenic mouse and examined their T cell expression profiles in detail as described in Materials and Methods. We found that eGFP was expressed in CD4SP CD69⁺ thymocytes as well as in other stages of both Tg^{43G17} mice and Tg^{7.1-*Ib.eGFP*} mice. In contrast, eGFP expression was essentially abolished when *TCE-7.1* was deleted, and expression reverted to the levels observed in non-transgenic mice (Fig. 4A and B and data not shown). Moreover, we found that the pattern of eGFP expression in Tg^{43G17} mice and Tg^{7.1-*Ib.eGFP*} mice reflected the expression of endogenous *Gata3* during late stages of thymocyte development in

TABLE 1. eGFP expression in the peripheral blood of F₀ Tg mice

Transgene	No. of mice with CD4 ⁺ eGFP ⁺ cells/no. of mice with Tg (PCR ⁺)
Tg ^{43G17}	7/8
Tg ^{43G17Δ7.1}	0/14
Tg ^{<i>Ib.eGFP</i>}	1 ^a /14
Tg ^{7.1-<i>Ib.eGFP</i>}	6/6

^a eGFP expression was detected in CD4⁺ cells and granulocytes (Gr1⁺Mac1⁺) in only one founder Tg^{*Ib.eGFP*} mouse. We concluded that it was ectopic expression since the remaining founder Tg^{*Ib.eGFP*} mice never expressed eGFP in CD4⁺ cells.

FIG. 4. A 7.1-kbp fragment (*TCE-7.1*) within the BAC overlap directs $\alpha\beta$ T cell reporter gene transcription. (A) eGFP expression in CD69⁺ CD4 SP thymocytes from Tg^{43G17}, Tg^{43G17Δ7.1}, Tg^{7.1-1b.eGFP}, Tg^{1b.eGFP}, and wild-type mice. Data are representative of results from at least three individual mice of each genotype. (B and C) Normalized mean fluorescence intensity (MFI) of eGFP in each population of thymocytes (B) and splenocytes (C). MFI was normalized using the LinearFlow green flow cytometry intensity calibration kit and presented as percentage of relative fluorescence of calibration beads. Error bars (B and C) denote means $\pm$ standard deviations (SD). Two lines of each construct-derived Tg mouse were examined (data not shown), and at least three individual mice of each Tg line were analyzed. Data represent a single line from each construct-derived Tg mouse. Note that all MFI expression data are presented on a log scale. ETP, lineage-negative (Lin⁻) CD25^{low} c-Kit^{high}; DN2, Lin⁻ CD25^{high} c-Kit^{high}; DN3, Lin⁻ CD25^{high} c-Kit^{low}; DN4, Lin⁻ CD25^{low} c-Kit^{low}; ISP, TCRbeta^{low} CD8 SP; CD69⁻ DP, TCRbeta^{low} CD69⁻ DP; CD69⁺ DP, TCRbeta⁺ CD69⁺ DP; CD4 SP CD69⁺, TCRbeta⁺ CD69⁺ CD4 SP; CD4 SP CD69⁻, TCRbeta⁺ CD69⁻ CD4 SP; CD8 SP CD69⁺, TCRbeta⁺ CD69⁺ CD8 SP; CD8 SP CD69⁻, TCRbeta⁺ CD69⁻ CD8 SP; Naïve CD4⁺, CD4⁺ CD62L^{high} CD44^{low}; Activated/Memory CD4⁺, CD4⁺ CD62L^{low} CD44^{high}; Naïve CD8⁺, CD8⁺ CD62L^{high} CD44^{low}; Activated/Memory CD8⁺, CD8⁺ CD62L^{low} CD44^{high}.

which positive selection and CD4 versus CD8 lineage choice occur.

Endogenous *Gata3* is induced by T cell receptor (TCR) signaling during positive selection at the DP stage, and this induction requires the activity of transcription factor c-Myb.

After DP cells differentiate into intermediate (CD69⁺) CD4 SP cells, endogenous *Gata3* expression remains high and then gradually diminishes during maturation into mature (CD69⁻) CD4 SP cells. In contrast, there is no induction of *Gata3* in intermediate (CD69⁺) CD8 SP cells, and its level of expression

diminishes even further as the cells differentiate into mature (CD69⁻) CD8 SP cells (18, 31, 34). We observed induced expression of the MFI of eGFP after cell differentiation from the preselection (CD69⁻ DP cells) stage into CD69⁺ DP cells, where positive selection has begun, in both Tg^{43G17} and Tg^{7.1-1b.eGFP} mice. eGFP increased in intermediate CD4 SP cells and declined thereafter in mature CD4 SP cells, as does endogenous *Gata3*. In CD8 SP cells, eGFP declined, again reflecting the endogenous *Gata3* expression pattern (Fig. 4B and data not shown). These results suggest that *TCE-7.1* contains *cis* information required to direct the late stages of *Gata3*-regulated thymocyte development.

In contrast to late thymocyte development, some differences in expression characteristics of the *TCE-7.1* transgenes were detected at earlier stages than the reported expression of endogenous *Gata3* mRNA (11, 44). For example, Tg^{7.1-1b.eGFP} and Tg^{43G17} mice displayed intense eGFP fluorescence at the ETP stage that gradually diminished as they differentiated into the DN2 and DN3 stages (Fig. 4B and data not shown). In addition, Tg^{7.1-1b.eGFP} CD69⁻ DP thymocytes expressed eGFP more intensely than immature CD8 SP (ISP) cells, while Tg^{43G17} exhibited no difference between those two populations. Neither is true of GATA-3 expression from the endogenous locus (Fig. 4B). Taken together, we tentatively conclude that the *cis* element(s) that is required to stimulate *Gata3* transcription at the DN3 stage (11, 44) or required to negatively regulate *Gata3* at the ETP stage must be located beyond the boundaries of the 43G17 BAC, while additional regulatory elements that negatively regulate *Gata3* at the CD69⁻ DP stage or positively regulate it at the ISP stage must exist outside *TCE-7.1* but may be included within the boundaries of BAC 43G17.

We also analyzed splenocytes to examine reporter gene expression in peripheral T cells in greater detail. Both Tg^{43G17} and Tg^{7.1-1b.eGFP} displayed higher eGFP MFI in naïve CD4⁺ cells than in naïve CD8⁺ cells, which is similar to the pattern of endogenous *Gata3*. Those activities were essentially ablated in the absence of *TCE-7.1* (Fig. 4C and data not shown). These results indicate that *TCE-7.1* is critical for transcription in peripheral T cells, although small differences were detectable. For example, eGFP was higher in naïve CD4⁺ cells than in activated/memory CD4⁺ cells of both Tg^{43G17} and Tg^{7.1-1b.eGFP} mice (Fig. 4C), which again does not perfectly reflect the *in vivo* changes in GATA-3 that occur during T cell differentiation. In addition, eGFP in naïve and activated/memory CD8⁺ splenocytes remained high compared to CD4⁻ CD8⁻ splenocytes, especially in Tg^{7.1-1b.eGFP} mice (Fig. 4C). Taken together, these results demonstrate that *TCE-7.1* is vital for transcription of a *Gata3* promoter-directed reporter gene in thymocytes and in splenocytes *in vivo*, although the element within *TCE-7.1* may not alone be sufficient to precisely recapitulate all aspects of *Gata3* T cell expression. We therefore speculate that additional regulatory elements that negatively regulate *Gata3* in peripheral CD8⁺ T cells may exist outside *TCE-7.1* but within the 43G17 BAC.

In order to determine whether *TCE-7.1* contains the element(s) specifying increased *Gata3* transcription in Th2 cells but not in Th1 cells (52, 53), we analyzed the expression of eGFP in CD4⁺ cells from Tg^{43G17} mice under a variety of cytokine stimulatory conditions (see Materials and Methods).

After stimulation, eGFP in CD4⁺ cells under Th2-polarizing and nonpolarizing conditions was not significantly altered, although the cells did express low levels of eGFP (Fig. 5), in keeping with the observed properties of GATA-3 expression *in vivo*. We confirmed that the *in vitro* polarization of these cells was successful by monitoring cytokine induction (IFN- γ and IL-4) (data not shown). In CD4⁺ cells recovered from Tg^{43G17 Δ 7.1} mice, eGFP was not observed. In contrast, Tg^{7.1-1b.eGFP} CD4⁺ cells cultured under stimulatory conditions somewhat surprisingly displayed greatly reduced eGFP fluorescence under Th2-polarizing or nonpolarizing conditions compared to that of naïve CD4⁺ cells. Moreover, Tg^{7.1-1b.eGFP} cells under Th1-polarizing conditions actually displayed higher eGFP expression than under Th2-polarizing or nonpolarizing conditions (Fig. 5 and data not shown). Both results contradict the GATA-3 expression characteristics observed *in vivo*. These data suggest that *TCE-7.1*, either in concert with the *Gata3-1b* promoter or even within the context of the entire BAC43G17 clone, does not contain the regulatory element that specifies *Gata3* activation in Th2 cells or in activated CD4⁺ T cells, although *TCE-7.1* does contain an element that is critical for high-basal-level transcription in stimulated CD4⁺ cells. Furthermore, a putative *cis* element that must be located outside the boundaries described by *TCE-7.1*, but within the boundaries specified by BAC 43G17, may additionally be required for the repression of *Gata3* in Th1 cells and naïve CD4⁺ T cells.

***TCE-7.1* bears *Gata3* T and NK cell-specific regulatory information.** In addition to its well-characterized roles in $\alpha\beta$ T cell, sympathoadrenal, kidney, parathyroid, breast epithelial, and epidermal development (5, 15, 21, 24, 25, 30, 32, 43), GATA-3 has been shown to play critical roles in the generation and maturation of NK cells (39, 46). In addition, GATA-3 is expressed in $\gamma\delta$ T cells (19) as well as in hematopoietic progenitors (38), although its function there is not well understood. We analyzed NK cells, $\gamma\delta$ T cells, and hematopoietic progenitors (lineage⁻ Sca1⁺ c-Kit^{hi} [LSK]) in multiple transgenic lines to ascertain whether *TCE-7.1* was also active in those related lymphoid lineages.

Somewhat surprisingly, robust eGFP fluorescence was observed in thymic NK cells and $\gamma\delta$ T cells in both Tg^{7.1-1b.eGFP} and Tg^{43G17} mice, while mice bearing the 7.1-kbp deleted BAC Tg^{43G17 Δ 7.1} or mice bearing only the *Gata3-1b* promoter failed to express eGFP (Fig. 6A). In contrast to GATA-3-expressing cells (e.g., $\alpha\beta$ or $\gamma\delta$ T cells or NK cells), other hematopoietic lineages that do not express endogenous GATA-3 also failed to express eGFP, in both Tg^{7.1-1b.eGFP} and Tg^{43G17} mice (Fig. 6A). Furthermore, neither Tg^{7.1-1b.eGFP} nor Tg^{43G17} express eGFP in the early hematopoietic progenitor compartment (Fig. 6B). These results demonstrated that *TCE-7.1* is active in T and NK cells but not in other hematopoietic lineages or progenitors.

In order to more globally examine whether the eGFP expression conferred by *TCE-7.1* was T cell specific, we examined Tg^{7.1-1b.eGFP} expression by IVIS Spectrum whole-body *in vivo* imaging. Robust eGFP fluorescence was detected exclusively in the thymi of Tg^{7.1-1b.eGFP} neonates (Fig. 7A). In contrast, other organs in these mice did not display expression above background levels; similarly, neither Tg^{1b.eGFP} nor wild-type mice expressed detectable eGFP (Fig. 7A and data not shown). *Ex vivo* imaging of individual adult organs confirmed the con-

FIG. 5. *TCE-7.1* directs reporter gene transcription in stimulated CD4⁺ cells. (A) Histograms of eGFP in naïve CD4⁺ splenocytes and stimulated CD4⁺ splenocytes under various conditions. Data are representative of results from at least three individual mice of each genotype. (B) MFI of eGFP in naïve and stimulated CD4⁺ splenocytes under various conditions. Note that MFI was not normalized using calibration beads in this experiment. Error bars denote means $\pm$ SD. Two lines of each construct-derived Tg mouse were examined (data not shown), and at least three individual mice of each Tg line were analyzed. Data represent a single line from each construct-derived Tg mouse. Note that MFI expression data are presented on a log scale.

FIG. 6. Among hematopoietic cells, *TCE-7.1* confers only NK cell and $\alpha\beta$ and $\gamma\delta$ T cell enhancer activity. (A) Normalized MFI of eGFP in erythroid cells (TER119⁺), B cells (CD19⁺ B220⁺ CD3⁻), and myeloid cells (Gr1⁺ Mac1⁺) in the bone marrow, as well as $\gamma\delta$ T cells (TCR $\gamma\delta$ ⁺) and NK cells (CD3⁻ CD19⁻ DX5⁺) in the thymus are shown. Error bars denote means $\pm$ SD. Note that MFI data are presented on a log scale. (B) eGFP expression in bone marrow hematopoietic progenitors (Lin⁻ Sca1⁺ c-Kit^{hi}). The shaded histograms indicate each Tg mouse, while dashed lines indicate wild-type mice. For both panels A and B, two lines of each construct-derived Tg mouse were examined (data not shown), and at least three individual mice of each Tg line were analyzed. Data represent a single line from each construct-derived Tg mouse.

clusions on the living neonatal mice. As shown in Fig. 7B, eGFP fluorescence was detected only in the thymus of Tg^{7.1-1b.eGFP} mice but not in other organs. In agreement with conclusions from the *in vivo* imaging, neither Tg^{1b.eGFP} nor wild-type adult mice displayed detectable eGFP expression (Fig. 7B). These results demonstrated conclusively that sequences within *TCE-7.1* direct exclusive T and NK cell-specific transcription of *Gata3*.

DISCUSSION

Here, we report that a 7.1-kbp DNA fragment (abbreviated *TCE-7.1*) located 280 kbp 3' to the *Gata3* gene contains *cis* information that is critical for the transcription of *Gata3*, both at multiple stages of T cell development and in thymic NK

cells. While previous experiments have identified central roles for GATA-3 at multiple stages of T cell development (19, 36, 37, 54), it is expressed at all stages that have been examined, but its level clearly differs markedly between developmental stages (e.g., in CD4 versus CD8 SP cells or in Th1 versus Th2 cells) (17, 18, 52, 53). Although several *trans*-acting factors are believed to directly regulate *Gata3* (1, 12, 31, 50, 51), a coherent mechanism explaining how this information is integrated to allow differential, stage-specific *Gata3* expression at multiple stages of T cell development *in vivo* has not emerged.

The BAC-trap transgenic assay utilized in this report reiterated the generality of this assay, showing that even a very distant *cis* element, located more than 200 kbp from the structural gene (the amount of information usually borne in a BAC), can be identified, thus revealing the position of a cell-specific enhancer that is capable of conferring transcription to a reporter gene at several discrete developmental stages, from ETP to peripheral CD4⁺ T cells, *in vivo*. The expression pattern of the reporter was similar to that of endogenous *Gata3* even though differences were documented. Since eGFP expression was not observed in the absence of the enhancer-bearing fragment, we conclude that this element is critical for transcription of the *Gata3* gene in T lymphocytes.

In this study, both Tg^{43G17} and Tg^{7.1-1b.eGFP} displayed expression profiles that reflected almost perfectly the endogenous *Gata3* expression pattern in late stages of thymocyte development. At the DP stage, the TCR α locus begins to rearrange, and subsequently a low level of the TCR $\alpha\beta$ complex is expressed. Those DP cells are poised for positive selection, although most of them fail to emerge intact from selection: only a few cells that have an appropriate TCR affinity are positively selected and finally emerge to differentiate into either CD4 SP or CD8 SP cells. Endogenous *Gata3* is induced at the onset of positive selection, and its expression is controlled by TCR signaling (18, 34). Based on the remarkably similar expression patterns of endogenous *Gata3*, Tg^{43G17}, and Tg^{7.1-1b.eGFP} during late thymocyte development, we conclude that *TCE-7.1* contains the activity required for development through those stages.

We found that *TCE-7.1* harbors multiple putative transcription factor binding sites through bioinformatic analyses (data not shown). Perhaps not surprisingly, candidate binding sites for many transcription factors that are critical for T cell development can be identified in this region. For example, highly species-conserved sequences contain putative binding sites for transcription factors c-Myb, Runx, E2A, and TCF-1 as well as others. The proto-oncogene c-Myb is required for the induction of *Gata3* following TCR signaling, and the binding of c-Myb to the *Gata3-1b* promoter is detectable in thymocytes (31). It is important to remember that this same Myb binding site is present in the B125 YAC, and that the YAC-derived transgene was not expressed in thymocytes. Moreover, this binding site was absent in all of the BAC and 7.1-1b.eGFP reporter constructs examined in this report, but nonetheless the reporter gene was induced in the T cells of both Tg^{7.1-1b.eGFP} and Tg^{43G17} mice. Since the fragment bearing the T cell enhancer activity contains several putative c-Myb binding sites, it is possible that these enhancer sites participate in *Gata3* induction by c-Myb.

Although expression in both the Tg^{7.1-1b.eGFP} and Tg^{43G17}

FIG. 7. The *TCE-7.1* enhancer is T cell specific. (A) eGFP expression in living P4 mice. Data are representative of multiple pups examined in two independent experiments. (B) eGFP expression in various organs from each genotype of adult mice. Three individual mice of each genotype were analyzed. B, brain; Li, liver; H, heart; K, kidney; Lu, lung; St, stomach; T, thymus; Sp, spleen. In both panels A and B, two lines of both *Tg 7.1-1b.eGFP* and *Tg 1b.eGFP* mouse were examined (data not shown).

mice resembled that of endogenous *Gata3*, whether this element or group of elements constitute a bona fide *cis* element for *Gata3* has not been conclusively demonstrated. Only two spliced ESTs other than *Gata3* are located within 300 kbp of *TCE-7.1*. Those two ESTs, *AK080422* and *AK035738*, have been identified as nonprotein-coding mRNAs (35). *AK080422* was detected in a mouse neonatal cerebellum cDNA library, while *AK035738* was observed in an adult male mouse urinary bladder cDNA library. The function of those two ESTs is unknown, and their expression in thymocytes has not been detected. Taken together, assigning *TCE-7.1* activity to the *Gata3* gene is likely the most conservative interpretation of these data.

Numerous interesting questions emerge from this study: does a single element within *TCE-7.1* control *Gata3* expression in $\alpha\beta$ T, $\gamma\delta$ T, and NK cells, or alternatively do multiple elements within *TCE-7.1* each regulate transcription in those distinct lineages? Do multiple elements, perhaps in different combinations, consort to elicit proper stage-specific *Gata3* activation during T cell development, or do different cofactors, all acting on a single *cis* element within *TCE-7.1*, function at different stages to confer the specificity? Answers to these fascinating questions should be resolved soon by the many groups studying *Gata3* function in T cell transcription.

Finally, the data predict that (one or multiple) sequences within *TCE-7.1* must collaborate with as-yet-undiscovered *cis* elements lying beyond the *TCE-7.1* boundaries to fully recapitulate proper *Gata3* expression in T lymphocytes. *TCE-7.1* clearly contains sequences that are important for directing transcription in both the T cell and NK cell lineages. Further analysis of *Gata3* gene regulation in T cells should help us to not only understand the complex mechanisms of gene regulation that are used to confer T cell specificity to this regulatory network but perhaps might also shed light on the mechanisms

where GATA-3 may play an oncogenic role in leukemia and lymphoma (34, 45, 48).

ACKNOWLEDGMENTS

We thank M. Sartor for advice in the bioinformatics analysis, I. Maillard for advice and critical reading of the manuscript, and W. Filipiak, M. Van Keuren, and the Transgenic Animal Model Core of the University of Michigan's Biomedical Research Core Facilities for the generation of some of these transgenic mice.

Core support was provided by the University of Michigan Cancer Center and the Center for Organogenesis. Initial support for this project was provided by the NIH (GM28896). None of the authors have competing financial interests.

REFERENCES

1. Amsen, D., et al. 2007. Direct regulation of *Gata3* expression determines the T helper differentiation potential of Notch. *Immunity* 27:89–99.
2. Reference deleted.
3. Anderson, M. K., et al. 2002. Definition of regulatory network elements for T cell development by perturbation analysis with PU.1 and GATA-3. *Dev. Biol.* 246:103–121.
4. Asnagli, H., M. Afkarian, and K. M. Murphy. 2002. Cutting edge: identification of an alternative GATA-3 promoter directing tissue-specific gene expression in mouse and human. *J. Immunol.* 168:4268–4271.
5. Asselin-Labat, M.-L., et al. 2007. Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. *Nat. Cell Biol.* 9:201–209.
6. Boyle, A. P., et al. 2008. High-resolution mapping and characterization of open chromatin across the genome. *Cell* 132:311–322.
7. Chen, D., and G. Zhang. 2001. Enforced expression of the GATA-3 transcription factor affects cell fate decisions in hematopoiesis. *Exp. Hematol.* 29:971–980.
8. Crawford, G. E., et al. 2006. DNase-chip: a high-resolution method to identify DNase I hypersensitive sites using tiled microarrays. *Nat. Methods* 3:503–509.
9. Crawford, G. E., et al. 2004. Identifying gene regulatory elements by genome-wide recovery of DNase hypersensitive sites. *Proc. Natl. Acad. Sci. U. S. A.* 101:992–997.
10. Crawford, G. E., et al. 2006. Genome-wide mapping of DNase hypersensitive sites using massively parallel signature sequencing (MPSS). *Genome Res.* 16:123–131.
11. David-Fung, E.-S., et al. 2006. Progression of regulatory gene expression states in fetal and adult pro-T-cell development. *Immunol. Rev.* 209:212–236.

12. Fang, T. C., et al. 2007. Notch directly regulates Gata3 expression during T helper 2 cell differentiation. *Immunity* **27**:100–110.
13. Fiering, S. N., et al. 1991. Improved FACS-Gal: flow cytometric analysis and sorting of viable eukaryotic cells expressing reporter gene constructs. *Cytometry* **12**:291–301.
14. George, K. M., et al. 1994. Embryonic expression and cloning of the murine GATA-3 gene. *Development* **120**:2673–2686.
15. Grigorieva, I. V., et al. 2010. Gata3-deficient mice develop parathyroid abnormalities due to dysregulation of the parathyroid-specific transcription factor Gcm2. *J. Clin. Invest.* **120**:2144–2155.
16. Hasegawa, S. L., et al. 2007. Dosage-dependent rescue of definitive nephrogenesis by a distant Gata3 enhancer. *Dev. Biol.* **301**:568–577.
17. Hendriks, R. W., et al. 1999. Expression of the transcription factor GATA-3 is required for the development of the earliest T cell progenitors and correlates with stages of cellular proliferation in the thymus. *Eur. J. Immunol.* **29**:1912–1918.
18. Hernández-Hoyos, G., M. K. Anderson, C. Wang, E. V. Rothenberg, and J. Alberola-Ila. 2003. GATA-3 expression is controlled by TCR signals and regulates CD4/CD8 differentiation. *Immunity* **19**:83–94.
19. Hosoya, T., et al. 2009. GATA-3 is required for early T lineage progenitor development. *J. Exp. Med.* **206**:2987–3000.
20. Hosoya, T., I. Maillard, and J. D. Engel. 2010. From the cradle to the grave: activities of GATA-3 throughout T-cell development and differentiation. *Immunol. Rev.* **238**:110–125.
21. Kaufman, C. K., et al. 2003. GATA-3: an unexpected regulator of cell lineage determination in skin. *Genes Dev.* **17**:2108–2122.
22. Khandekar, M., N. Suzuki, J. Lewton, M. Yamamoto, and J. D. Engel. 2004. Multiple, distant Gata2 enhancers specify temporally and tissue-specific patterning in the developing urogenital system. *Mol. Cell. Biol.* **24**:10263–10276.
23. Ko, L. J., et al. 1991. Murine and human T-lymphocyte GATA-3 factors mediate transcription through a *cis*-regulatory element within the human T-cell receptor delta gene enhancer. *Mol. Cell. Biol.* **11**:2778–2784.
24. Kouro-Mehr, H., E. M. Slorach, M. D. Sternlicht, and Z. Werb. 2006. GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. *Cell* **127**:1041–1055.
25. Kurek, D., G. A. Garinis, J. H. van Doorninck, J. van der Wees, and F. G. Grosveld. 2007. Transcriptome and phenotypic analysis reveals Gata3-dependent signalling pathways in murine hair follicles. *Development* **134**:261–272.
26. Lakshmanan, G., K. H. Lieu, F. Grosveld, and J. D. Engel. 1998. Partial rescue of GATA-3 by yeast artificial chromosome transgenes. *Dev. Biol.* **204**:451–463.
27. Lakshmanan, G., et al. 1999. Localization of distant urogenital system-, central nervous system-, and endocardium-specific transcriptional regulatory elements in the GATA-3 locus. *Mol. Cell. Biol.* **19**:1558–1568.
28. Lee, E. C., et al. 2001. A highly efficient Escherichia coli-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. *Genomics* **73**:56–65.
29. Lieu, K. H., G. Li, Y. Zhou, F. Grosveld, and J. D. Engel. 1997. Temporal and spatial control of murine GATA-3 transcription by promoter-proximal regulatory elements. *Dev. Biol.* **188**:1–16.
30. Lim, K. C., et al. 2000. Gata3 loss leads to embryonic lethality due to noradrenaline deficiency of the sympathetic nervous system. *Nat. Genet.* **25**:209–212.
31. Maurice, D., J. Hooper, G. Lang, and K. Weston. 2007. c-Myb regulates lineage choice in developing thymocytes via its target gene Gata3. *EMBO J.* **26**:3629–3640.
32. Moriguchi, T., et al. 2006. Gata3 participates in a complex transcriptional feedback network to regulate sympathoadrenal differentiation. *Development* **133**:3871–3881.
33. Nawijn, M. C., et al. 2001. Enforced expression of Gata-3 in transgenic mice inhibits Th1 differentiation and induces the formation of a T1/ST2-expressing Th2-committed T cell compartment in vivo. *J. Immunol.* **167**:724–732.
34. Nawijn, M. C., et al. 2001. Enforced expression of GATA-3 during T cell development inhibits maturation of CD8 single-positive cells and induces thymic lymphoma in transgenic mice. *J. Immunol.* **167**:715–723.
35. Okazaki, Y., et al. 2002. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature* **420**:563–573.
36. Pai, S.-Y., M. L. Truitt, and L.-C. Ho. 2004. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. *Proc. Natl. Acad. Sci. U. S. A.* **101**:1993–1998.
37. Pai, S.-Y., et al. 2003. Critical roles for transcription factor GATA-3 in thymocyte development. *Immunity* **19**:863–875.
38. Sambandam, A., et al. 2005. Notch signaling controls the generation and differentiation of early T lineage progenitors. *Nat. Immunol.* **6**:663–670.
39. Samson, S. L., et al. 2003. GATA-3 promotes maturation, IFN-gamma production, and liver-specific homing of NK cells. *Immunity* **19**:701–711.
40. Taghon, T., et al. 2001. Enforced expression of GATA-3 severely reduces human thymic cellularity. *J. Immunol.* **167**:4468–4475.
41. Taghon, T., M. A. Yui, and E. V. Rothenberg. 2007. Mast cell lineage diversion of T lineage precursors by the essential T cell transcription factor GATA-3. *Nat. Immunol.* **8**:845–855.
42. Taylor, J., et al. 2006. ESPERR: learning strong and weak signals in genomic sequence alignments to identify functional elements. *Genome Res.* **16**:1596–1604.
43. Tsarovina, K., et al. 2010. The Gata3 transcription factor is required for the survival of embryonic and adult sympathetic neurons. *J. Neurosci.* **30**:10833–10843.
44. Tydell, C. C., et al. 2007. Molecular dissection of prethymic progenitor entry into the T lymphocyte developmental pathway. *J. Immunol.* **179**:421–438.
45. van Hamburg, J. P., et al. 2008. Cooperation of Gata3, c-Myc and Notch in malignant transformation of double positive thymocytes. *Mol. Immunol.* **45**:3085–3095.
46. Vosshenrich, C. A., et al. 2006. A thymic pathway of mouse natural killer cell development characterized by expression of GATA-3 and CD127. *Nat. Immunol.* **7**:1217–1224.
47. Xi, H., et al. 2007. Identification and characterization of cell type-specific and ubiquitous chromatin regulatory structures in the human genome. *PLoS Genet.* **3**:e136.
48. Xu, W., and B. L. Kee. 2007. Growth factor independent 1b (Gfi1b) is an E2A target gene that modulates Gata3 in T-cell lymphomas. *Blood* **109**:4406–4414.
49. Yamamoto, M., et al. 1990. Activity and tissue-specific expression of the transcription factor NF-E1 multigene family. *Genes Dev.* **4**:1650–1662.
50. Yang, X. O., et al. 2009. Requirement for the basic helix-loop-helix transcription factor Dec2 in initial Th2 lineage commitment. *Nat. Immunol.* **10**:1260–1266.
51. Yu, Q., et al. 2009. T cell factor 1 initiates the T helper type 2 fate by inducing the transcription factor GATA-3 and repressing interferon-gamma. *Nat. Immunol.* **10**:992–999.
52. Zhang, D. H., L. Cohn, P. Ray, K. Bottomly, and A. Ray. 1997. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J. Biol. Chem.* **272**:21597–21603.
53. Zheng, W., and R. A. Flavell. 1997. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* **89**:587–596.
54. Zhu, J., et al. 2004. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. *Nat. Immunol.* **5**:1157–1165.