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Cutting Edge: Identification of an Alternative GATA-3 Promoter Directing Tissue-Specific Gene Expression in Mouse and Human[1]

Hélène Asnagli, Maryam Afkarian, and Kenneth M. Murphy[2]

The GATA family of transcription factors regulates development of multiple tissues. Several GATA factors have two promoters directing distinct tissue-specific expression. Although GATA-3 acts in both neuronal and thymocyte development, no alternative promoter usage has been reported. We examined various cell types and tissues for potential alternative GATA-3 transcripts and identified an alternative transcript directed by a promoter located 10 kb upstream of the recognized promoter. Sequences within this promoter and alternative first exon are highly conserved between mouse and human genomes. This new promoter is expressed selectively in the brain but is essentially undetectable in the thymus. In contrast, the recognized promoter is selectively expressed in the thymus but not in the brain. We also observed a gradual increase in expression from this new promoter during Th2 development. These results indicate that similar to other GATA factors, the *GATA-3* gene can be controlled by two promoters that may direct lineage- and tissue-specific expression. ***The Journal of Immunology,*** **2002, 168: 4268–4271.**

In embryonic development, GATA-3 is expressed in several tissues including placenta, central and peripheral nervous systems, liver, and thymus (1, 2); but in the adult, expression is only in thymocytes, T cells, and CNS. Murine *GATA-3* deficiency is lethal (3) due to noradrenaline deficiency (4). Human *GATA-3* haploinsufficiency causes a DiGeorge-like syndrome with parathyroid, auditory, kidney, and craniofacial abnormalities (5). *GATA-3* is required in thymocyte maturation (6, 7). Thus, *GATA-3* acts in development of several tissues.

Polarization of $CD4^+$ T cells into distinct cytokine-secreting subsets is controlled by various transcription factors, with GATA-3 inducing Th2 cytokines and inhibiting Th1 cytokines (8). In naive $CD4^+$ T cells, the TCR and JAK/STAT signaling pathways influence GATA-3 expression (9). Initially low GATA-3 expression is augmented on T cell activation by IL-4 and decreased by IFN-γ and IL-12. Ectopic GATA-3 expression induced full Th2 development even in $Stat6^{-/-}$ T cells (10). Thus, GATA-3 expression is also critical for commitment to the Th2 phenotype of $CD4^+$ Th cells.

GATA-3 is the only GATA factor expressed in T cells. Yet ectopic expression of GATA-1, -2, and -4 into naive $CD4^+$ T cells also induced Th2 development in Stat6-deficient T cells (11) but worked by inducing endogenous GATA-3 (10, 11). Thus, T cells appear to regulate GATA-3 expression both by an IL-4/Stat6-dependent pathway and by a separate GATA-dependent pathway, suggesting two distinct patterns of transcriptional regulation for GATA-3. The GATA family gene organization is highly conserved (12, 13). Murine *GATA-1* (14), mouse and human *GATA-2* (13, 15), chicken *GATA-5* (16, 17), and *GATA-6* (18) each contains two distinct promoters and alternate first exons that independently regulate gene expression in distinct tissues or cell lineages.

This study describes two modes of GATA-3 expression in T cells, an IL-4-dependent mode operating in naive $CD4^+$ T cells and an IL-4-independent mode operating in Th2 effector cells. Despite the lack of reported alternative promoters described for GATA-3 (1, 12, 19), we asked whether alternative promoter usage could underlie these dual modes of GATA-3 expression. In this report, we describe the identification of an alternative GATA-3 promoter and first exon that is conserved between human and mouse genomic sequences. We find that these two promoters are used differentially between brain and thymus and between naive and fully differentiated Th2 cells. Therefore, this report establishes an important physical basis for differential regulation of GATA-3 expression in distinct tissues and during distinct phases of Th2 development.

Materials and Methods

Reagents

DO11.10 $\alpha\beta$ TCR-transgenic mice (9, 20), murine rIL-4, and rIL-12 (21) have been described. Anti-CD28 (37.51) and CD3 (500A2) were gifts from Dr. J. P. Allison (Berkeley, CA) and were purified by affinity chromatography from culture supernatant or used as ascites.

T cell differentiation and restimulation

Sorted $CD4^+Mel\text{-}14^{high}$ DO11.10 T cells were stimulated with 0.3 μM OVA, under Th1 or Th2 conditions as described (10). The Th0 condition refers to the priming of T cells with Ag/APCs in the presence of neutralization Abs against IL- 4 (11B11) (22), IL-12 (Tosh) (23), and IFN-γ (H22) (24). Naive or differentiated T cells were reactivated with plate-bound anti-CD3 (1/1000) and anti-CD28 (1 μg/ml) for 48 h, and RNA was prepared.

Department of Pathology and Immunology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110

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[2] Address correspondence and reprint requests to Dr. Kenneth M. Murphy, Department of Pathology and Immunology, Howard Hughes Medical Institute, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110. E-mail address: murphy@immunology.wustl.edu

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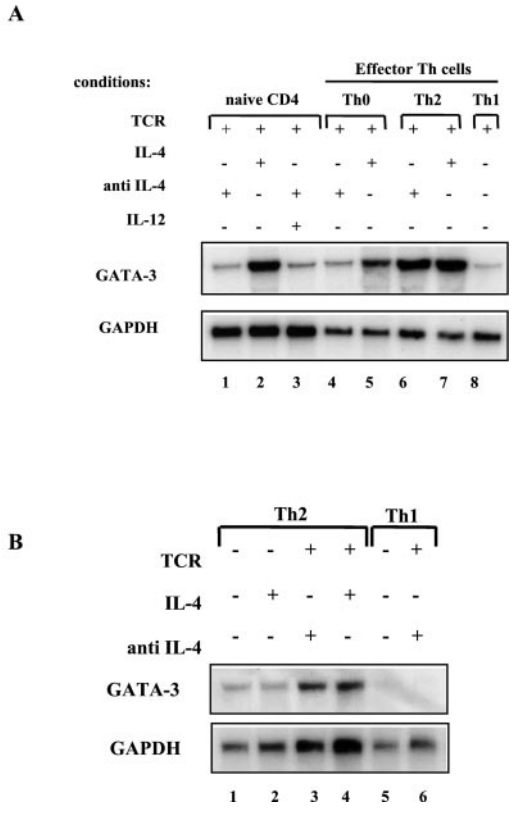

FIGURE 1. Distinct modes of GATA-3 gene expression exhibited by naive and differentiated T cells. *A,* For naive T cells (*lanes 1–3*), $CD4^{+}Mel\text{-}14^{high}$ T cells were sorted from DO11.10 TCR-transgenic mice and activated as described (9) with the additions (+) of cytokines or Abs. Anti-IFN-γ (H22), 5 μg/ml, was included in all conditions. Anti-IL-12 (1:10) was added to all conditions except where IL-12 cytokine was added (*lane 3*). For effector analysis (*lanes 4–8*), T cells were primed either in Th0 conditions (anti-IL-12, 1/10 (23); anti-IL-4, 1/10 (22), and anti-IFN-γ (H22), 5 μg/ml (24), or in Th1 or Th2 conditions (9). For effector T cells, cells were harvested on day 7 and restimulated with plate-bound anti-CD3 Ab (TCR) (1/1000) in the presence (+) of the indicated cytokines or Abs. After 48 h, cells were harvested and analyzed by Northern blot. *B,* Th1 or Th2 effector cells as described in *A* were restimulated on day 7 after primary stimulation and restimulated with (+) or without (−) plate-bound anti-CD3 Ab (TCR), with (+) or without (−) addition of IL-4 or anti-IL-4 Ab.

RNA, Northern blots

GATA-3 Northern analysis was done as described (9). RNA tissues from thymus and brain were from Ambion (Austin, TX).

5′-RACE and RT-PCR

Total RNA was prepared from Th0 or Th2 effector cells 48 h after stimulation with Ag/APC. 5′-RACE was conducted using the SMART RACE kit (Clontech Laboratories, Palo Alto, CA). GATA-3 primers were against the murine GATA-3 exon 2 (1): primer RT, CGATGTTAAAAAGTACG TCCACCTCT, for the reverse transcription reaction; primer b, CTTT GCGGGATAGTTTAGCAA, for the PCR amplification reaction (Fig. 3*A*). PCR fragments were cloned into pGEM-T Easy vector (Promega, Madison, WI) and screened by Southern blot with primer b to identify exon 2. We excluded exon 1 using an exon 1-specific probe, generated by PCR using the following primer pair: GPE1, GAGTAGCAAGGAGCGTA GAGGAGG (1); and primer c, GAACACTGAGCTGCCTGGCGCCGT, an exon 1-specific antisense primer. Clones hybridizing to the exon 2 but not to the exon 1 probe were sequenced.

Expression analysis by RT-PCR

For GATA-3 exon 1a, the primer pairs were primer a, CATCAGCCAG GTTTTACC, and exon 2 antisense primer b (above). For GATA-3 exon 1b, primer c and primer b above were used. PCR conditions were denaturation at 94°C for 4 min, followed by 20–35 cycles at 94°C for 20 s, 60°C for 20 s, and 72°C for 2 min, with final elongation at 72°C for 4 min using the *Taq* polymerase (Promega). PCR products were analyzed by Southern blot with ^{32}P-end-labeled oligonucleotides: for GATA-3 exon 1a, primer 1a-AS, CAGCTGGTTTGGTTTTTGTTTTTTT; for GATA-3 exon 1b, primer 1b-AS, GAGTAGCAAGGAGCGTAGAGGAGGA (Fig. 3*A*).

For comparisons of human and murine genomic sequences, we used the Celera Discovery System (San Francisco, CA).

Results and Discussion

We examined first the requirements for GATA-3 induction in naive T cell and at different stages of Th development. We first compared GATA-3 mRNA expression by Northern analysis in naive T cells or T cells that had undergone 1 wk of development under various conditions (Fig. 1*A*). In naive T cells, a very low level of *GATA-3* gene expression was observed upon activation in the absence of IL-4 and in the presence of IL-12 (Fig. 1*A*, *lanes 1–3*).

Upon TCR stimulation, significant GATA-3 induction occurred in the presence of IL-4 compared with TCR alone (Fig. 1*A*, *lane 2* compared with *lane 1*) or to IL-12 treatment (Fig. 1*A*, *lane 3* consistent with earlier results) (9, 25). This result indicates that IL-4 signaling is required for strong GATA-3 induction in naive T cells.

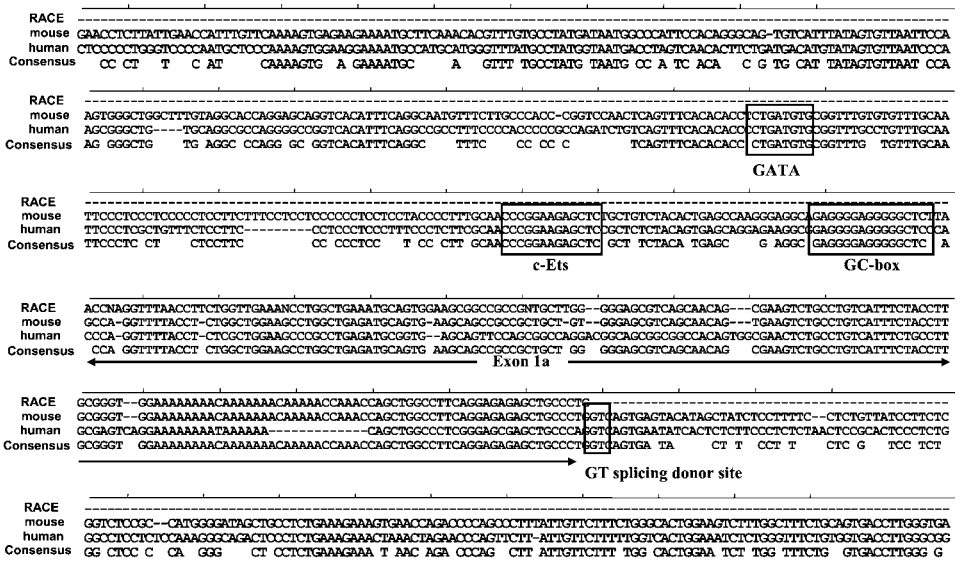

FIGURE 2. Conservation of murine and human GATA-3 exon 1a and flanking sequences. The sequence of the exon 1a derived above (RACE) was aligned with homologous sequences identified from the murine and human genome databases from the Celera Discovery System. Sequence analysis for potential *cis*-regulatory elements was performed using Transfac resources (27).

A similar requirement was found in T cells that were initially primed for 1 wk in the absence of IL-4 (Th0 conditions) and restimulated on day 7 (Fig. 1*B*, *lanes 4* and *5*). Thus, naive T cells and T cells not previously exposed to IL-4 each require IL-4 signaling for strong induction of GATA-3 expression.

In contrast, T cells previously exposed to IL-4 such as Th2 cells exhibited a distinct pattern of GATA-3 inducibility (Fig. 1*A*, *lanes 6* and *7*). In resting Th2 cells, GATA-3 mRNA was expressed and was inducible by TCR. Further, addition of IL-4 during TCR signaling did not augment GATA-3 induction. Finally, IL-4 treatment of resting Th2 cells did not induce GATA-3 expression (Fig. 1*B*). Therefore, in differentiated Th2 cells, TCR signaling induces GATA-3 expression without an apparent requirement for IL-4. In summary, naive T cells clearly require both TCR and IL-4 signaling to induce GATA-3 expression, but differentiated Th2 cells can induce GATA-3 in response to TCR signaling alone.

Because several GATA family members have two alternative promoters that provide distinct patterns of gene expression, we were interested in directly testing for the existence of an alternative promoter and first exon for GATA-3. Not knowing which tissues or conditions would use such a transcript, we obtained RNA from T cells activated under a variety of conditions including TCR-stimulated effector Th2 cells and TCR plus IL-4-stimulated Th0 cells. 5′ RACE analysis was done with reverse transcription of mRNA primed either with oligo(dT) or exon 2-specific primers. We identified two classes of 5′ RACE products. The first represented the published GATA-3 transcript using the recognized GATA-3 promoter and first exon (1). A second product class contained 3′-GATA-3 exon 2 sequences but had a unique 5′ 195-nucleotide sequence, similar in size to other recognized first exons of GATA factors. We used a bacterial artificial chromosome-containing murine *GATA-3* gene as a genomic template for PCR analysis using primers specific for this novel sequence. PCR with these primers generated a specific 9.5-kb product (data not shown). Thus, we have identified an putative novel exon for GATA-3 located ~10 kb upstream of the recognized exon 1. In this study, we refer to the previously recognized exon 1 as exon 1b and to the new exon 1 as exon 1a.

We compared the murine and human genomic sequences (Fig. 2). A blast search using the murine exon 1a (RACE) against the murine genome identified a region of 99% homology on mouse chromosome 2 located 9.4 kb upstream of GATA-3 exon 2, consistent with our genomic PCR analysis. A blast search using the murine exon 1a against the human genome identified a region of 96% homology on human chromosome 10 positions located 9.4 kb upstream of the human GATA-3 exon 2. Thus, exon 1a is conserved between human and murine *GATA-3* genes in a configuration similar to the conserved upstream exons described for *GATA-1* (14) and *GATA-2* (13, 15) genes. We also compared the genomic flanking sequences surrounding exon 1a for both murine and human genomes. A conserved GT dinucleotide is found immediately downstream of the human and murine transcribed exon 1a sequences, suggesting a conserved splice donor site for exon 1a. Also, the 200 nucleotides upstream of exon 1a are very highly conserved between murine and human, particularly a GC box, an E-26-binding site, and a nonconsensus GATA site which are perfectly conserved between the human and murine genomes, suggesting potential regulatory elements (Fig. 2).

To test for any differences in expression of exons 1a and 1b, we developed an exon-specific RT-PCR analysis (Fig. 3*A*). RT-PCR was conducted using oligo(dT)-primed cDNA templates followed by specific PCR amplification using primer pairs specific for exon 1a or 1b with a common primer specific to exon 2 (Fig. 3*A*). PCR products were identified by hybridization to exon-specific nested oligonucleotide probes (Fig. 3*A*). As positive controls, we included cell lines known to express GATA-3, including murine BW51.47 and EL4 thymomas and the murine neuroblastoma cell line C1300 (Fig. 3*B*). As a negative control, we analyzed the fibroblast cell line NIH3T3. Both murine thymomas strongly expressed both exon 1a and exon 1b. C1300 expressed both exon 1a and exon 1b but at significantly reduced levels. NIH3T3 cells were negative for both exons by RT-PCR

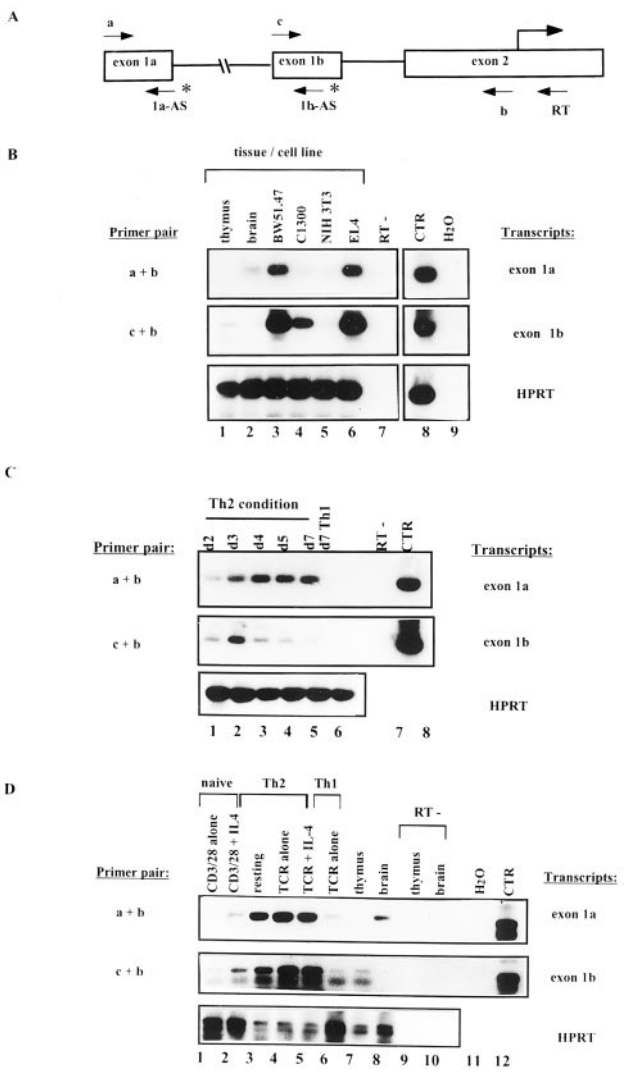

FIGURE 3. Differential usage of GATA-3 exons 1a and 1b in mouse tissue and cell lines. *A*, Primers used for RT-PCT analysis. Primers a and c are specific for the 1a and 1b exons. Primer b is antisense to exon 2 and is used in both reactions. Primers 1a-AS and 1b-AS are antisense to exons 1a and 1b, respectively, and are used for hybridization to PCR products. *B*, RT-PCR analysis of exons 1a and 1b. RNA is from the indicated cells. Controls are shown either with no reverse transcriptase (*lane 7*), no RNA (*lane 9*) or positive control with plasmids (*lane 8*). *C*, RNA from T cells under Th2 differentiation was extracted at the indicated time course point, and RT-PCR was performed using the primers shown in *A*. *D*, RNA was harvested from naive cells (*lanes 1* and *2*) after 48 h stimulation with coated anti-CD3/CD28 in presence of Abs against IL-4 and IFN-γ (*lane 1*) or in presence of IL-4 with Ab against IFN-γ (*lane 2*). RNA from day 7 Th2 (*lanes 3–5*) or Th1 (*lane 6*) cells was harvested after 48 h of restimulation with splenic APCs and anti-IFN-γ, anti-IL-12 and IL-4 or anti-IL-4 Abs as indicated. As controls, RNA from thymus or brain in the presence or absence of reverse transcriptase (RT) reverse transcription as indicated (*lanes 7–10*), no RNA (*lane 11*), or plasmid-positive control (*lane 12*).

as expected. All PCR products reflected expression by RNA, as controls lacking RT treatment failed to generate these products (Fig. 3*B, lanes 7* and *9*; Fig. 3*C, lanes 9* and *10*; Fig. 3*D, lanes 7*).

Next we examined differential exon usage in the thymus and brain tissues from adult mice. We found clear evidence for distinct exon usage in these tissues. Whole murine thymus mRNA was weakly positive for exon 1b but essentially negative for expression of exon 1a (Fig. 3*B*, compare *lane 1*, *upper* and *middle panels*; Fig. 3*D, lane 7*, compare *upper* and *middle panels*). In contrast, whole brain mRNA was positive for exon 1a but negative for detectable usage of exon 1b (Fig. 3*B, lane 2*; Fig. 3*D, lane 8*). Thus, whereas in vitro-adapted cell lines show no distinct alternative exon usage, whole tissues clearly indicate a differential usage of GATA-3 exons 1a and 1b between thymus and brain.

We also examined the potential for differential exon usage in T cells at different stages of Th cell development. Naive $CD4^+$ T cells were primed in vitro with anti-CD3 and anti-CD28 Abs in either the presence or absence of IL-4 and analyzed at 48 h after activation (Fig. 3*D*). In the absence of IL-4, exon 1b transcripts were only weakly detectable, and exon 1a transcripts were essentially undetectable (Fig. 3*D, lane 1*), whereas in the presence of IL-4, transcripts from both exon 1a and exon 1b were detected (Fig. 3*D, lane 2*). By comparison, fully differentiated Th2 cells strongly expressed exons 1a and 1b. Transcripts from both exons were detected in resting cells and were moderately induced on stimulation through the TCR activation in either the presence or absence of IL-4 (Fig. 3*D, lanes 3* and *5*). By contrast, TCR-stimulated Th1 cells showed very weak usage of either transcript (Fig. 3*C, lanes 6*; Fig. 3*D, lane 6*). Finally whole thymus RNA showed selective usage of exon 1b, whereas whole brain RNA showed selective usage of exon 1a. Interestingly, there appeared to be a gradual shift is usage during the time course of Th2 development from naive $CD4^+$ T cells from day 2 to day 7 after primary activation (Fig. 3*C*). Here, exon 1a, which is absent in thymus, was weakly expressed at 2 days of Th2 development but gradually increased to a maximum at day 7. By contrast, exon 1b, which is expressed in thymus, showed a peak at day 3 followed by a gradual decrease over the same time period.

In summary, we report the existence of a novel alternative GATA-3 promoter/exon located 10 kb upstream of the recognized GATA-3 exon 2 that is conserved between mouse and human, bringing *GATA-3* gene organization into a pattern recognized for several other GATA factors. Even though tissue culture adapted cell lines showed usage of both exons, examination of freshly isolated thymus and brain tissues indicated a distinct differential pattern of exon usage. A previous study attempted to identify GATA-3 transcripts by primer extension analysis of the murine BW5147 thymoma and MEL erythroleukemia but did not included nontransformed T cell or tissue (1). Interestingly, exon 1a is located near a previously identified DNase-hypersensitive site that is present in BW5147 but not in C1300 (26).

Important developmental roles for GATA-3 are recognized both in the sympathetic nervous system and in T cells, so that our finding of selective exon 1a expression in brain tissue and exon 1b in thymus fits into the paradigm of alternative promoter usage in distinct developmental contexts. In T cells, GATA-3 is also regulated by different pathways at distinct stages of Th2 development, suggesting a role for these two promoters here as well. It is important next to examine differential promoter usage in vivo during embryogenesis and during stages of Th2 development, which will likely require the generation of selective gene-targeting approaches to mark the usage of each promoter individually.

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