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From the cradle to the grave: activities of GATA-3 throughout T-cell development and differentiation

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Summary: GATA family transcription factors play multiple vital roles in hematopoiesis in many cell lineages, and in particular, T cells require GATA-3 for execution of several developmental steps. Transcriptional activation of the *Gata3* gene is observed throughout T-cell development and differentiation in a stage-specific fashion. GATA-3 has been described as a master regulator of T-helper 2 (Th2) cell differentiation in mature CD4⁺ T cells. During T-cell development in the thymus, its roles in the CD4 versus CD8 lineage choice and at the β -selection checkpoint are the best characterized. In contrast, its importance prior to β -selection has been obscured both by the developmental heterogeneity of double negative (DN) 1 thymocytes and the paucity of early T-lineage progenitors (ETPs), a subpopulation of DN1 cells that contains the most immature thymic progenitors that retain potent T-lineage developmental potential. By examining multiple lines of *in vivo* evidence procured through the analysis of *Gata3* mutant mice, we have recently demonstrated that GATA-3 is additionally required at the earliest stage of thymopoiesis for the development of the ETP population. Here, we review the characterized functions of GATA-3 at each stage of T-cell development and discuss hypothetical molecular pathways that mediate these functions.

Keywords: GATA-3, ETP, β -selection, CD4 SP, Th2

T-cell development in thymus

T-cell production in the thymus is supported by continuous, life-long migration of T-cell progenitors derived from hematopoietic stem cells (HSCs) in the bone marrow (1). Most if not all current models for lineage specification from HSCs derive from the concept that, as cells mature, they progressively lose more and more differentiation potential (Fig. 1). HSC and multi-potential progenitors (MPPs) reside in the phenotypically defined LSK [lineage⁻ (Lin⁻)Sca1⁺cKit^{hi}] population (Table 1), and early developmental induction of cell surface fms-like tyrosine kinase 3 (Flt3) expression within this population is accompanied by a loss of self-renewal potential (2, 3). In comparison to MPPs, lymphoid-primed MPPs (LMPPs) have markedly reduced erythroid potential

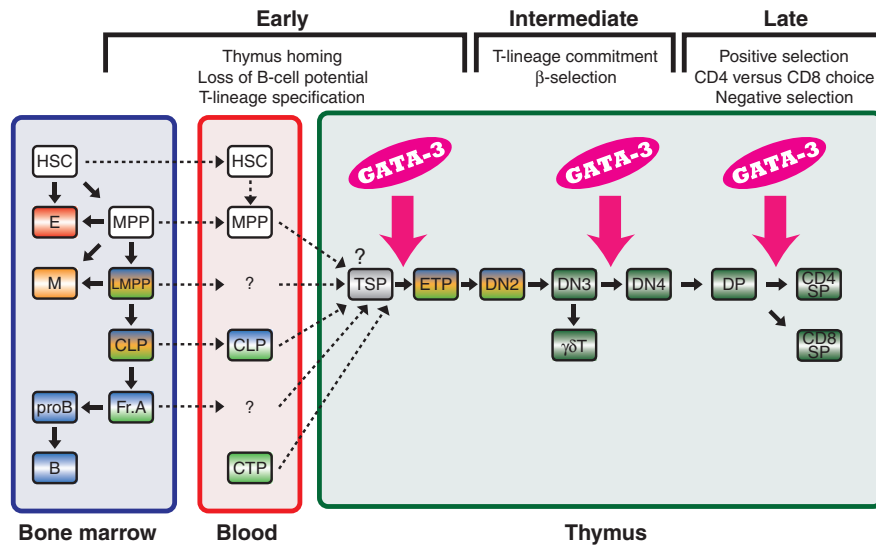


Fig. 1. A model for T lymphopoiesis. Popular models for lineage specification from HSCs derive from the concept that, as cells mature, they progressively lose differentiation potential. Myeloid, B and T-lineage potential are shown in orange, blue, and green, respectively. Cells that are committed to the T lineage are shown in dark green. The identity of the thymus homing cells remains controversial. Possible homing cells are shown in dashed lines. GATA-3 is required for the development of ETP, DN4, and CD4 SP T cells in the thymus (magenta arrow). E, erythroid lineage cells; M, myeloid lineage cells; B, B lymphoid lineage cells.

Table 1. Hematopoietic populations and abbreviations (mouse)

Cell population	Abbreviation	Cell surface phenotype
Lineage negative cells	Lin ⁻	
Hematopoietic stem cell	LSK	Lin ⁻ Sca1 ⁺ cKit ^{hi}
Multipotent progenitors	HSC	CD150 ⁺ CD48 ⁻ CD34 ⁻ Flt3 ⁻ LSK
Lymphoid-primed multipotent progenitors	MPP	Flt3 ^{lo} LSK
Common lymphoid progenitors	LMPP	Flt3 ^{hi} LSK
Early thymic progenitors	CLP	Lin ⁻ IL7Rα ⁺ Sca1 ^{lo} cKit ^{lo}
	ETP	CD8 ⁻ CD3 ⁻ TCRβ ⁻ γδTCR ⁻ NK1.1 ⁻ CD11c ⁻ Mac1 ⁻ Gr1 ⁻ B220 ⁻ CD19 ⁻ Ter119 ⁻ cKit ^{hi} CD25 ⁻
Double negative T cells, stage 1	DN1	CD4 ⁻ CD8 ⁻ CD44 ⁺ CD25 ⁻
Double negative T cells, stage 2	DN2	Either Lin ⁻ cKit ^{hi} CD25 ⁺ or Lin ⁻ CD44 ⁺ CD25 ⁺
Double negative T cells, stage 3	DN3	Either Lin ⁻ cKit ^{lo/-} CD25 ⁺ or Lin ⁻ CD44 ⁺ CD25 ⁺
Double negative T cells, stage 4	DN4	Either Lin ⁻ cKit ^{lo/-} CD25 ⁻ or Lin ⁻ CD44 ⁺ CD25 ⁻
Double positive	DP	CD4 ⁺ CD8 ⁺
Single positive	SP	Either CD4 ⁺ CD8 ⁻ or CD4 ⁻ CD8 ⁺

(4–7). While most hematopoietic lineage cells (specifically erythroid, myeloid, and B lymphoid cells and all of their progenitors) develop in the fetal liver and/or bone marrow, T lymphoid cells develop in the thymus, which provides unique microenvironmental cues that are essential for T-cell development (8).

T-lymphocyte progenitors migrate into the thymus from the fetal liver or bone marrow through the blood stream. Chemokines, cell adhesion molecules, and their receptors CCL25/CCR9, CCL19-CCL21/CCR7, and P-selectin/PSGL-1 regulate this migration (9–15). The identity of thymus-homing cells continues to be controversial (reviewed in 16). These cells have been proposed to reside in several different bone marrow compartments that are distinguishable by their expression of different cell surface markers: LSK (17–19), Lin⁻cKit^{lo}

Sca1^{lo}IL7Rα⁺ [common lymphoid progenitor (CLP)] (20, 21), and in the B220⁺CD19⁻ population (22, 23). Regardless of their cellular origin, very rare (and at this point, hypothetical) thymus-seeding progenitors (TSPs) upon encountering the thymic epithelium are thought to amplify to generate early T-lineage progenitors (ETPs), the most immature T-cell progenitors in the thymus that have been characterized (24–26).

Intrathymic ETPs constitute a cKit^{hi}Lin⁻ fraction that harbors all of the most potent T-cell developmental potential but comprises only a subset of about 2–4% of all double negative 1 (DN1) stage cells (27). Very shortly after thymic entry, these progenitors lose their B-cell potential and become specified to the T-cell lineage. The most immature subset (Flt3⁺) of adult ETPs retains modest B-cell developmental potential (27, 28). Thymic epithelial cells express the Notch ligand Delta-

like 4 and activate Notch1-mediated signals, which in turn promote T-cell development while at the same time repressing further B and myeloid cell development (27–32). However, the existence of T-restricted progenitors has also been reported to emerge from multiple other physiological sites: in fetal and adult peripheral blood (33, 34), in the fetal liver (35, 36), in the spleen shortly after bone marrow transplantation (37, 38), and even in the normal adult mouse spleen (39). In addition, inactivation of the transcriptional repressor LRF in HSCs activates high levels of Notch signaling in these cells and leads to the emergence of T-cell development in the bone marrow as well as in other extrathymic tissues (40). These data, taken together, suggest that prethymic T-lineage commitment may indeed occur at reduced incidence or under certain unusual circumstances but that T-cell development in tissues other than the thymus is actively suppressed under non-pathological conditions.

ETPs progress through the DN2 stage to the DN3 stage of development. While ETPs and DN2 cells retain myeloid lineage potential, DN3 cells have lost that potential and become fully committed to the T lineage (41, 42). During these intermediate stages of T-cell development, the *Tcr γ* , *Tcr δ* , and *Tcr β* loci undergo rearrangement (43, 44). At the same time, pre-T-cell antigen receptor- α (*Ptcr α*) and *Cd3 ϵ* transcripts increase (45, 46). CD3, TCR β , and pre-TCR α make up the pre-TCR complex (reviewed in 47). Cells that have formed a functional pre-TCR complex can develop into double positive (DP) cells, while cells that have failed to initially produce a functional complex rearrange the other *Tcr β* allele in a second attempt to generate a functional pre-TCR complex; if rearrangements on both alleles fail to generate an active TCR β protein, those cells are eliminated by apoptosis. This step is referred as the β -selection checkpoint and is essential for the development of $\alpha\beta$ T cells. Complex transcriptional inputs, including from RBPJ, MYB, TCF1, LEF1, E2A, HEB, GFI1, IKAROS, RUNX/CBF β , PU.1, and GATA-3 form a network under the direct and indirect influence of Notch signaling to support T-cell specification and commitment from multi-potential progenitors (reviewed in 48). Other essential players include cytokines and their receptors on hematopoietic cells, such as interleukin 7 (IL7)/IL7R, stem cell factor (SCF)/cKit, Flt3 ligand, and Flt3 (27, 49–54).

The cells that survive β -selection develop into DN4 cells and then into immature single positive (CD8⁺CD4⁻) and DP stages of development. DP cells undergo rearrangement of the *Tcr α* locus, and this rearrangement results in the formation of the mature TCR $\alpha\beta$ complex. Next, the DP cells develop into either CD4⁻CD8⁺ (CD8 SP) or CD4⁺CD8⁻ (CD4 SP) cells, or

alternatively are eliminated by apoptosis. This CD8 versus CD4 lineage choice determines mature T-cell fate and is controlled by an intricate interplay between an increasingly well understood transcription factor network, including the GATA-3, TOX, Th-POK, and RUNX, all under the influence of TCR signaling (55–66). Those cells bearing TCR complexes that are able to bind to MHC survive (positive selection), while other cells are eliminated by apoptosis (death by neglect). A second round of negative selection at the DP and SP stages eliminates by apoptosis the developing T cells that respond to self-antigens.

Immature T-cell migration through the thymus ensures an orderly progression of T-cell development by providing non-cell-autonomous cues in discrete subregions of the thymus (reviewed in 67). Mature CD4 SP or CD8 SP T cells exit the thymus and home through the bloodstream to secondary lymphoid organs, where mature naive lymphocytes are maintained and where adaptive immune responses are initiated. Cytokines, their signaling pathways, and tissue-restricted transcription factors form an elaborately orchestrated network that maintains the proper continuous production of T cells. GATA-3 is one of the essential factors for T-cell development and differentiation, and its importance has been demonstrated from the beginning (in thymic ETPs) to the end of T-cell life (in peripheral Th2 CD4⁺ T cells).

Transcription factor GATA-3 is vital for T-cell development

At the time we originally cloned GATA-3, we found that it was the sole member of the GATA zinc-finger-type transcription factor family expressed in T lymphocyte cells (68). Six GATA factors have been identified in mammals (68–71), and all members appear to bind to a WGATAR recognition sequence found in the promoters and/or enhancers of literally thousands of tissue-restricted genes (72, 73). Hematopoietic cells (as do some additional tissues) express the ‘hematopoietic’ factors: GATA-1, GATA-2, and GATA-3. Each tissue and cell lineage expresses only very specific GATA factors, and only at very specific stages of maturation or development (Table 2). GATA-1 is essential for the development of erythroid cells, for the proliferation and/or maturation of megakaryocytes, for the production of circulating blood platelets, for the differentiation of eosinophils, and for mast cell differentiation (74–78). GATA-2 is required for proliferation/survival of early hematopoietic progenitors as well as for mast cell formation (79–82). GATA-3 is vital for the development of T cells in thymus (56, 57, 83–85), Th2 differentiation of peripheral CD4⁺ T cells (86–99),

Table 2. Expression of GATA factors in various hematopoietic lineages

Hematopoietic progenitors	GATA-1, GATA-2, GATA-3
Erythroid cells	GATA-1
Megakaryocytes	GATA-1, GATA-2
Mast cells	GATA-1, GATA-2
Eosinophils	GATA-1
T cells	GATA-3
NK cells	GATA-3

maturation of bone marrow natural killer (NK) cells (100), and development of thymic NK cells (101).

GATA-2 and GATA-3 are also expressed and play key developmental roles in a wide variety of non-hematopoietic tissues. GATA-3 is essential for norepinephrin biosynthesis in sympathetic ganglia and the adrenal gland, and consequently *Gata3* null mutant fetuses die at around e11 of a secondary cardiac insufficiency (102–104). This was definitively shown to be the case by rescuing *Gata3* null mutant embryos to birth by feeding of pregnant intercrossed dams with catecholamine intermediates (103, 105). GATA-3 has also been shown to participate in development of the Wolffian duct and metanephric kidney (103, 104, 106), in hair follicle development and skin cell lineage differentiation (107, 108), in development of the luminal epithelium of the mammary gland (109, 110), as well as in the differentiation of multiple tissues derived from the pharyngeal arches, such as the jaw, parathyroid gland, and thymus (103, 111).

Expression of GATA-3 during T-cell development

During hematopoiesis, the *Gata3* gene is first transcriptionally activated in HSCs, defined variously as either IL7R α ⁻Thy1.1^{lo}LSK (112) or CD38⁺CD34⁻LSK (113) or Thy1.1^{lo}LSK (114) or CD34⁻Flt3⁻LSK (7). The VCAM1⁺Flt3^{hi} sub-fraction of MPPs expresses *Gata3* mRNA (6). CLPs (112, 115) and pre-pro-B cells (Hardy fraction A) (115), which retain both T-cell and B-cell developmental potential, also express *Gata3* mRNA in the bone marrow. In the thymus, *Gata3* mRNA is expressed in the earliest ETPs that have been identified (27). *Gata3* mRNA levels gradually increase between the ETP and DN3 stages and slightly diminish again in DN4 cells (46, 116). At the DP stage, *Gata3* mRNA and protein are both repressed and then *Gata3* transcription is strongly induced in late DP stage and remains high at the CD4 SP stage, while diminishing in CD8 SP cells (56, 84, 117).

GATA-3 is required at very early stages of T-cell development

The involvement of GATA-3 during early stages of thymopoiesis was first suggested in 1996 following the analysis of

hematopoietic cells that had diminished GATA-3 expression as a result of antisense experiments (118). These investigators introduced antisense oligonucleotides into e13 Lin⁻cKit⁺ fetal liver cells or thymocytes and examined their development into Thy1⁺ cells in fetal thymus organ culture. The introduction of antisense oligonucleotide into fetal liver progenitors resulted in diminished Thy1⁺ cell development to <20% of control (nonsense oligonucleotide-transfected) cells. At the same time, the introduction of antisense oligonucleotides into fetal cKit⁺ thymocytes resulted in an approximately 50% reduction in Thy1⁺ cell development. Although alternative explanations were possible, the greater T-cell attenuation observed among the transfected fetal liver progenitors suggested that GATA-3 might be important for T lymphopoiesis, even before thymic entry.

Additional *in vivo* evidence for the involvement of GATA-3 in early T-cell development was provided in experiments that generated blastocyst chimeras starting with *Gata3*-null embryonic stem (ES) cells (either *Gata3*^{-/-} null or control *Gata3*^{+/-} heterozygous ES cells, both carrying a LacZ knock-in allele) (84). The contribution of the mutant cells was detected using infused fluorescein di- β -D-galactopyranoside (FDG), a substrate that fluoresces upon cleavage by β -galactosidase. These investigators observed 2.2% FDG⁺ cells in DN1 thymocytes of *Gata3*^{+/-} heterozygous ES chimera, but only 0.4% in *Gata3*^{-/-} (null mutant) ES chimeras. The data indicated that the contribution of *Gata3*^{+/-} heterozygous ES cells to the T-cell lineage was about 10% in blastocyst chimeras, as compared to 50–70% in the rest of the body (heart, brain, kidney, and lungs). Furthermore, the contribution of *Gata3*^{-/-} null ES cells to the DN1 population was even lower, suggesting a dosage-sensitive requirement for GATA-3 in DN1 thymocytes. However, since relatively abundant NK1.1⁺ cells are also contained within the DN1 fraction (27, 85, 119) in which ETPs are sparse (2–4% of DN1 cells) (27), and given the fact that GATA-3 activity is known to be required for thymic NK cell development (101), the data were sufficiently equivocal that it was impossible to conclude decisively that GATA-3 was required for the development of early T-cell progenitors.

To gain more precise insight into the earliest stage at which GATA-3 becomes required for T-cell development prior to the β -selection checkpoint, we examined the development of immature T cells in multiple *Gata3* mutant backgrounds: in *Gata3* hypomorphic mutant embryos, in irradiated adult mice reconstituted with *Gata3* null or hypomorphic mutant hematopoietic progenitor cells, and in adult mice in which the *Gata3* gene was conditionally ablated (85). In reconstituted mice, *Gata3*^{z/z} (null) mutant hematopoietic cells showed normal

contributions to the bone marrow HSC, LMPP, CLP and Fr.A early hematopoietic compartments, all of which retain T-cell developmental potential. In contrast, the absolute number of $Gata3^{z/z}$ ETPs in HSC-repopulated recipient mice was <10% of heterozygous controls. In those reconstituted animals, development of DN2 and later stage T cells was even more significantly impaired, to <1% of controls. Consistent with these adoptive transfer experiments, conditional compound mutant ($Gata3^{flox/flox};Tg^{Mx1cre}$) mice bearing activated Cre generated fewer than 3% of normal ETP numbers when compared to control ($Gata3^{flox/+};Tg^{Mx1cre}$) Cre-activated animals.

To examine the possible direct involvement of GATA-3 in the development of fetal ETPs, we took advantage of $Gata3$ hypomorphic mutant ($Gata3^{g/g}$) mice. Earlier experiments showed that GATA-3 is expressed during embryogenesis in the third pharyngeal pouch, from which the thymic epithelium develops (our unpublished observations). While drug-rescued $Gata3$ null mutant embryos develop no detectable thymus (103), homozygous $Gata3$ hypomorphic mutants indeed develop small thymi, and within those hypomorphic $Gata3^{g/g}$ thymi, embryos generated 16% or 13% of normal ETP numbers when compared to wildtype at e15.5 or e18.5, respectively.

Enforced expression of GATA-3 by retroviral transduction has been shown to induce cKit expression (45, 120), and so to ask whether the observed reduction in mutant ETP and DN2 cells might be a simple consequence of reduced cKit cell surface expression, we analyzed the number of $Lin^{-}DN1$ cells in $Gata3$ mutant animals. Indeed, careful gating on Lin^{-} thymocytes has been shown to enrich for *bona fide* ETPs within the DN1 population (27). The absolute number of those $Lin^{-}DN1$ cells was reduced in both the $Gata3^{g/g}$ hypomorphic mutant embryos and in Cre-induced ($Gata3^{flox/flox};Tg^{Mx1cre}$) $Gata3$ conditionally mutant animals (85). The summary data consistently demonstrated that GATA-3 is required for the development of ETPs, while at the same time it appears to be dispensable for the development of prethymic T-cell progenitors.

How does GATA-3 control ETP development? Since we observed no developmental impairment in prethymic progenitors but a severe reduction in the earliest $Gata3$ null T-cell population, we considered four possibilities: that GATA-3 controls (i) homing of prethymic progenitors into the thymus; (ii) the differentiation of TSPs to ETPs; (iii) ETP proliferation, and/or (iv) ETP viability. Since $Gata3$ mutant fetal livers generated fewer $Lin^{-}CD27^{+}cKit^{hi}CD25^{-}$ cells (corresponding to prethymic lymphoid progenitors plus thymic ETPs) (17, 121) *in vitro* in OP9-DL1 co-cultures, we tentatively concluded that a homing deficiency was unlikely to be a primary cause

of the reduced ETP numbers observed in the $Gata3$ mutants (85). However, we have not directly examined the thymus homing potential of $Gata3$ mutant progenitor cells, so a role for GATA-3 in thymus homing remains possible.

Blood-borne thymic seeding progenitors (TSPs) are thought to be extremely rare and their exact identity is unknown. The most immature subset of adult ETPs expresses the Flt3 cytokine receptor (27). To determine whether the reduced number of ETPs in the $Gata3$ mutants was caused by a differentiation arrest from early to late ETP subsets, we hoped to analyze the development of early ETP (Flt3⁺ ETP) and late ETP (Flt3⁻ ETP) in adoptive transfer recipients. Unfortunately the very low frequency of ETP recovery from the mutants (<10% of the normal adult ETP population) precluded recovery of consistent, reliable data. Analysis of $Gata3^{g/g}$ hypomorphic mutant cells in adoptive transfer recipients should be a more useful approach in this regard.

To address the third possibility, we examined the cell cycle status of $Gata3$ mutant cells. The percentage of e18.5 $Gata3^{g/g}$ ETP in S phase was very slightly higher than in $Gata3^{+/+}$ ETP (our unpublished data). To examine the cell-cycle status in the complete absence of GATA-3, we again employed OP9-DL1 co-cultures. When compared to wildtype $Gata3$, we again observed a slightly higher percentage of cells in S phase among $Gata3^{z/z} Lin^{-}CD27^{+}cKit^{hi}CD25^{-}$ cells. Thus, decreased cycling also cannot explain the dearth of ETPs in $Gata3$ -deficient mice.

We also examined the viability of $Gata3$ mutant T-cell progenitors. Since rapid clearance of dead cells by phagocytes in the thymus (122) might conceal small differences in cell longevity, we analyzed the viability of mutant cells in OP9-DL1 co-cultures by Annexin V staining. We observed no increase in the percentage of Annexin V⁺ cells in comparison to wildtype progenitors. Taken together, these data deductively suggest that the major GATA-3 cell-autonomous role in thymopoiesis is to control the differentiation of TSPs to ETPs and other downstream progenitors rather than participating in cell survival or proliferation, although further studies will be required to confirm or refute this hypothesis.

Since GATA-3 has been implicated in the control of differentiation at early stages of T-cell development, one might wonder if enforced expression of GATA-3 in multipotent prethymic progenitors might trigger precocious or unusually robust T-lineage development. However, the opposite was found to be true. Retroviral overexpression of GATA-3 in mouse bone marrow Thy1.1^{lo}LSK cells reportedly induced megakaryocyte and erythroid differentiation (123). Forcibly overexpressed GATA-3 by retroviral infection of mouse fetal

liver progenitors caused impaired development of DN2, DN3, and DP T cells in fetal thymus organ culture (45). In a third study, retrovirally forced expression of GATA-3 in mouse fetal liver LSK or dedicated lymphoid progenitors was reported to block the development of DN2 and DN3 T cells in OP9-DL1 co-cultures (120). These results collectively suggest that GATA-3 activity must be kept low in prethymic progenitors in order to induce the initiation of T-cell fate and that the activation of Notch signaling, which is able to promote T-cell development (29, 124), cannot overcome the repressive effects of non-physiologically elevated levels of GATA-3.

Gata3 null mutant ES cells contribute to both B and myeloid cells in ES chimeric mouse bone marrow (83), and *Gata3* null mutant e11.5 fetal liver cells generated similar numbers of B220⁺ cells as control progenitors in OP9 co-cultures (125). *Gata3* null and *Gata3* hypomorphic mutant hematopoietic progenitor cells showed neither increased nor decreased development of B or myeloid cells in the bone marrow and peripheral blood in adoptive transfer experiments (85). Furthermore, *Gata3* hypomorphic embryos displayed normal development of B, myeloid, and erythroid cells in progenitors recovered from e18.5 fetal liver or spleen (85). These data demonstrate that GATA-3 is dispensable for the development of all B-lineage and myeloid cells.

GATA-3 is required at intermediate stages (DN2 to DN4) of T-cell development

The contribution of GATA-3 to intermediate stages of T-cell development has been demonstrated by conditional *Gata3* ablation in DN2-DN3 thymocytes using the *Lck* promoter to direct the expression of Cre recombinase (*Gata3^{flox/flox};Tg^{Lckcre}*) (57). This mutant mouse accumulated abnormally abundant DN3 cells and a reduced number of DN4 and later stage T cells. *Gata3^{flox/flox};Tg^{Lckcre}* DN3 thymocytes rearranged the *Tcrb* gene, but DN4 cells had less intracellular TCR β protein than wildtype and an increased percentage of Annexin V⁺ apoptotic cells (57). These data suggest that *Gata3* deficient cells fail to form sufficient pre-TCR complex and are eliminated by apoptosis. A transgenic rescue attempt with DO11.10, which carries an major histocompatibility complex (MHC) class II-restricted rearranged TCR, failed to rescue reduced DP and SP T cells in the mutants (57). This failure suggested that GATA-3 must play an unidentified role in addition to the pre-TCR complex formation for the progression from the DN3 to DN4 stage. In contrast to thymocytes at the adult stage, the introduction of ROG (purported to be a natural inhibitor of GATA-3 in Th2 cells) (126), into mouse e14.5 fetal thymocytes (composed mostly of DN1-DN3 cells that

have not yet been subject to β -selection) had little effect on TCR β ⁺ cell numbers in fetal thymus organ cultures (56). In our studies, *Gata3^{g/g}* hypomorphic embryos had a normal DN4/DN3 profile compared to controls, but *Gata3^{g/g}* cells indeed showed developmental defects at the adult DN3 to DN4 transition in adoptive transfer recipients (85). These data suggest that β -selection is less sensitive to reduced GATA-3 protein abundance in fetal than in adult thymi. Successful rearrangement of *Tcrd* and *Tcrg* genes at the DN2-DN3 stage is also necessary for the generation $\gamma\delta$ T cells in the thymus. GATA-3 is expressed in $\gamma\delta$ T cells (85), but its requirement or role in thymic $\gamma\delta$ T cells is not known.

Forced expression of GATA-3 in human CD34⁺CD3⁻CD4⁻CD8⁻ immature thymocytes (prior to *Tcrb* gene rearrangement) (127) caused enhanced initial development toward the DP stage in fetal thymus organ cultures, while inducing a severe reduction in thymic cellularity after day 10 (128). In contrast, retroviral forced expression of GATA-3 in e14.5 to e15.5 DN1 and DN2 thymocytes inhibited differentiation into DP cells in fetal thymus organ cultures and in OP9-DL1 co-cultures (45, 120). In the absence of Notch signaling, forced expression of GATA-3 in mouse DN1 and DN2 fetal thymocytes promoted mast cell differentiation, a latent intrinsic potential of normal DN1 and DN2 thymocytes (120). Under normal physiological conditions, both GATA-2 and GATA-1 are expressed in mast cells and play essential roles in their differentiation (78, 80). Since GATA family proteins can apparently all bind to the GATA core DNA element (72, 73) and have been shown to be at least partially compensatory (129, 130), it is possible that more abundant GATA-3 generated in the forced expression models drives ectopic expression of genes that are under the control of either GATA-1 or GATA-2. We conclude that finely tuned levels of GATA-3 activity are required to survive the β -selection step in adult thymi. In support of this argument, we have shown previously that transcriptional regulation can be exquisitely sensitive to the absolute abundance of a transcription factor *in vivo* (131).

GATA-3 is required at late stages (DP to SP) of T-cell development in the thymus

GATA-3 expression is re-induced between early DP and late DP stages and remains high at the CD4 SP stage, while diminishing in CD8 SP T cells (56, 84, 117). It was found that forced transgenic expression of GATA-3 throughout T-cell development, achieved by placing it under the control of the human CD2 promoter/LCR (132, 133), did not impede thymocyte development but induced apoptosis and inhibited the final maturation of CD8 SP T cells in the thymus (134). Forced

expression of GATA-3 in e16.5 thymocytes that were enriched for DP cells resulted in reduced generation of abundant TCR cell surface expression and increased CD4/CD8 ratios in fetal thymus organ cultures (56). Mice that were conditionally ablated for *Gata3* at the DP stage and beyond using a Cd4-directed Cre transgene (*Gata3^{flox/flox}:Tg^{Cd4cre}*) generated reduced numbers of CD4 SP cells but normal numbers of CD8 SP cells (57). Inhibition of GATA-3 by retroviral introduction of ROG or anti-GATA-3 shRNAs into e14.5 to e16.5 thymocytes decreased the percentage of CD4 SP cells among the transduced TCR β^{hi} cells that emerged from fetal thymus organ cultures (56). These results demonstrate that GATA-3 is required for CD4 SP cell development after positive selection.

GATA-3 promotes and is required for Th2 differentiation of peripheral CD4⁺ T cells

Peripheral naive helper CD4⁺ T cells differentiate into several subsets that produce distinct patterns of cytokines (reviewed in 135, 136). GATA-3 is highly expressed in Th2 cells, but not in Th1 cells (86, 87). Enforced expression of GATA-3 by either retroviral infection or transgenic means promotes Th2 differentiation and inhibits Th1 differentiation (88, 92, 94), while inhibition of *Gata3* using either small interfering RNA (siRNA) or conditional *Gata3* deletion significantly limits Th2 differentiation (95, 96, 98). Furthermore, GATA-3 plays roles in the chromatin modification of Th2 cytokine loci, including the *Il4*, *Il5*, and *Il13* genes (90, 91, 93, 97, 99). These data indicate that GATA-3 is a master regulator of Th2 differentiation.

Target genes of GATA-3 in T cells

GATA-3 is presumed, or in a few cases known, to control T-cell development by *trans*-activation of various stage-specific target genes. What are the functional targets of GATA-3 during development from prethymic progenitors to ETPs? Although this is of course a critical question since this is the first currently documented stage to require GATA-3 activity in early hematopoiesis, no conclusive data are available so far from GATA-3 loss- or gain-of-function experiments. The very low number of ETPs and developmental arrest produced at or before ETP stages in *Gata3* mutants make it difficult to discriminate loss of direct control by GATA-3 from loss of certain cell populations in the absence of GATA-3. The gain-of-function experiments (45, 120, 123) do not appear to have identified target genes that would explain the crucial importance of GATA-3 in these early stages.

During intermediate stages of T-cell development, one important cluster of target genes that are regulated by GATA-3

is the Tcr genes. We and others have shown that GATA-3 binds to enhancer and promoter elements of the *Tcrb*, *Tcrg*, and *Tcrd* genes (137–139). However, adult mice that were conditionally ablated for *Gata3* at the DN2-DN3 transition (in *Gata3^{flox/flox}:Tg^{Lckcre}* mice) showed normal levels of *Tcrb* mRNA but reduced intracellular TCR β protein in DN3 and DN4 cells (57). These data suggest that GATA-3 may somehow regulate TCR β expression post-transcriptionally. GATA-3 binds to the *Rag2* promoter in gel mobility shift assays (140), but conditionally mutant *Gata3* mice show normal rearrangement of the *Tcrb* gene (57), indicating that GATA-3 is dispensable for *Tcrb* gene rearrangement. A transgenic rescue attempt with DO11.10, which carries an MHC class II-restricted rearranged TCR, failed to rescue reduced DP and SP T cells in the mutants (57). This failure suggested that GATA-3 must control a currently unidentified gene(s) that is critical for the development from the DN3 to DN4 stage.

To identify transcriptional target genes of GATA-3 prior to β -selection, GATA-3 was forcibly expressed by recombinant retroviral infection of e14.5 fetal thymocytes (which are principally composed of DN cells that have not yet undergone β -selection), and changes in gene expression were analyzed 24 h postinfection (45, 120). Abundant ectopic expression of GATA-3 in these progenitor T cells activated *Hes1*, a direct target of the Notch signaling pathway that is known to be important for DN cell development (141) and at the same time represses the *Sfp1* gene encoding PU.1. PU.1 gradually diminishes in abundance from the DN1 to DN3 stages and has been shown to block T-cell development when forcibly expressed in T-lymphocyte progenitors (142). Based on the short-term response to Notch signaling, PU.1 and GATA-3, Georgescu et al. (143) proposed a basic gene regulatory network during the DN stages. It will be important to provide new inputs and insights to this proposed framework and to experimentally assess the relationships amongst these factors. In contrast, forced expression of GATA-3 in T-cell progenitors exerted little effect on many other genes that are important for T-cell development, including *Il7r*, *Ptcr*, *Rag1*, *Rag2*, *CD3e*, *Myb*, *Tcf7*, *Lef1*, *E2a*, *Gfi1*, *Ikzf1*, *Runx1*, and *Notch1* (45, 120). Remarkably, forced GATA-3 expression also activated mast cell genes such as *cKit*, *Gata1*, *Gata2*, *Mitf*, *Cpa3*, and *Tal1* (120). Although *Hes1* was induced, an overall increase in Notch signaling was not observed after forced expression of GATA-3, because *Dtx1* transcripts (another downstream target of Notch signaling pathway) (144) were reduced in this experiment (45, 120). However, forced expression of GATA-3 by recombinant retroviral infection of bone marrow cells isolated from 5-FU treated mice induced *Dtx1* (145). Since *Dtx1* and *Dtx2* compound

knock-out mice have no defect in T-cell development (146), any putative role for GATA-3 in *Dtx1* expression needs to be analyzed more carefully at each T-cell developmental step (assuming that the *Dtx1* mutant animals are indeed true nulls) (147). In addition to gain-of-function experiments, loss-of-function experiments will be essential to identify *bona fide* GATA-3 targets. In this regard, the *Gata3* hypomorphic mutant mice (85) should prove to be invaluable, since ETP development is reduced but not completely missing in these mice and the development of the hypomorphic DN4 cells from DN3 cells is compromised in adoptive transfer animals.

During the CD4 versus CD8 lineage choice, GATA-3 directly regulates the *Zbtb7b* gene, which encodes the zinc finger transcription factor Th-POK (66). Transgenic forced expression of Th-POK in DP and SP T cells under the control of the human CD2 promoter/LCR inhibited CD8 SP cell development and caused re-direction of MHC class I-restricted CD8 SP cells to differentiate into CD4 SP cells. Furthermore, the loss of DNA binding activity of Th-POK caused by point mutation of the zinc finger coding sequence in the *Zbtb7b* gene redirected MHC class II-restricted CD4 SP cells to differentiate into CD8 SP cells (60, 61). Since transgenic expression of Th-POK failed to rescue the defect in CD4 SP cell development that is observed in the absence of *Gata3* (66), a gene(s) in addition to Th-POK that regulates CD4 SP cell development downstream of GATA-3 is likely to exist. Although GATA-3 binds to upstream regulatory sequences of the *Cd8a* gene (148), neither cell surface protein analysis nor reverse transcriptase polymerase chain reaction revealed any change in expression of CD8 or CD4 in *Gata3* mutant DP cells (56, 57).

During Th2 activation, GATA-3 induces the expression of Th2 cytokine genes (*Il4*, *Il5*, and *Il13*) coincident with multiple changes in chromatin modification (86, 87, 90, 91, 93, 97, 99). GATA-3 induces the *Bhlhe41* gene that encodes the basic helix-loop-helix transcription factor DEC2, which is specifically expressed in Th2 cells, is essential for Th2 differentiation, and promotes the expression of Th2 cytokines (149). GATA-3 also restricts Th1 differentiation by inhibiting *Stat4* transcription (88, 150) and directly represses *Ifng* (99). Chromatin immunoprecipitation (ChIP) experiments showed that GATA-3 binds to conserved non-coding sequences of the Th1-specific *Il18r1* gene in Th2 cells (151). Forced GATA-3 expression in naive CD4⁺ T cells from wildtype or *Il4*-deficient mice represses *Foxp3* mRNA, the key transcription factor gene in regulatory T cells (152). Similar repression is observed in human naive CD4⁺ CD45RA⁺ T cells, and ChIP experiments revealed the binding of GATA-3 to a palindromic GATA site of the *FOXP3* gene in Th2 cells but not in induced

Treg cells (153). These data initially indicate that *Foxp3* might be directly repressed by GATA-3.

In attempting to identify direct targets of GATA-3 and TBET in human Th1 and Th2 cells, GATA-3 was immunoprecipitated in ChIP assays followed by hybridization of the recovered DNA to microarrays containing probes for 8 kbp surrounding the transcription start sites of 18,450 protein-coding genes (ChIP-Chip) (154). This experiment identified 344 GATA-3-bound genes, including both Th2 and Th1. Thus, although GATA-3 target genes are beginning to be characterized in peripheral Th2 cells, the functionally important targets of GATA-3 remain enigmatic during the early, intermediate, and late stage of T-cell development in the thymus, with only a few exceptions (Fig. 2). Systematic identification of these downstream target genes will be required to understand these regulomes.

Upstream regulators of *Gata3* expression in T cells

An equally important unanswered question is the nature of the factor(s) that act upstream of *Gata3* and control its expression throughout T-cell development. Is *Gata3* gene activation spurred by a single predominant factor using a single enhancer throughout T-cell life? Or, do multiple factors drive *Gata3* gene expression at each stage through individually discrete stage-specific enhancers? Although information is not yet available to answer this question, the currently scarce emerging data suggests the involvement of several factors that act directly or indirectly to regulate *Gata3* at each developmental stage.

Activation of the Notch signaling pathway in hematopoietic progenitors induces T-cell development and multiple gene expression changes that are important for early and intermediate stages of T-cell development (29, 124). In the absence of Notch signaling, ETPs are not generated, while LSK progenitors circulating in the peripheral blood develop normally (27). Taghon et al. (121) performed a detailed kinetic study of fetal liver progenitors after exposure to Notch ligands in the OP9-DL1 co-culture system. Under these conditions, DN2 and DN3 T cells first emerge at days 3 and 4, respectively. Induction of *Gata3* mRNA was first observed on day 3 in parallel with elevated transcription from the *Tcf7*, *Ptcr1*, and *Cd3e* genes, while upregulated expression of the Notch target genes *Hes1* and *Hes5* was apparent even on day 1. Since *Gata3* mRNA increases gradually between the DN1 and DN3 stages (46, 116), these data suggest that increasing *Gata3* mRNA correlates with T-cell development but not in direct response to Notch signaling. Downregulation of the RUNX/CBF β complex activity impairs the generation/expansion of ETPs, as well as their differentiation into DN2 and DN3 cells (155). Exposure of

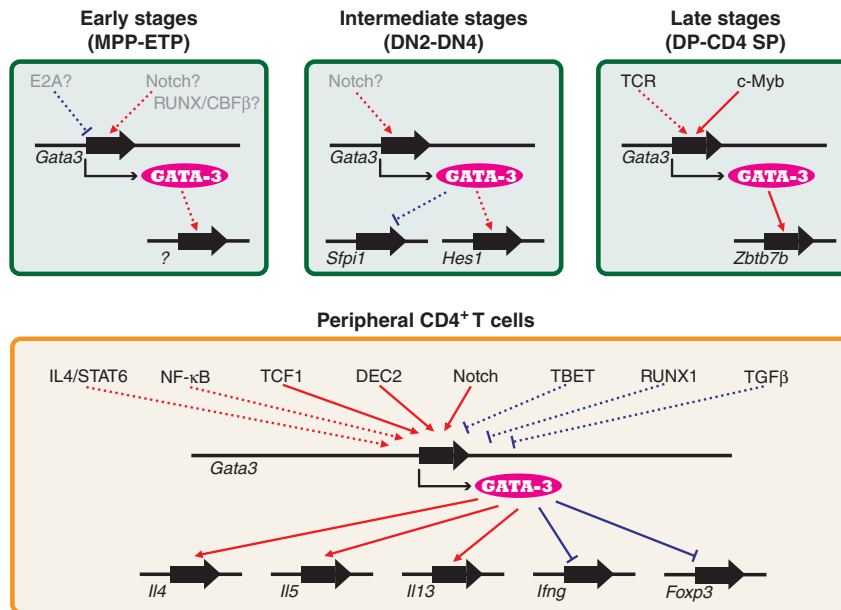


Fig. 2. A hypothetical transcriptional hierarchy mediated through and by GATA-3. Factors that regulate *Gata3* gene at each stage are shown. Positive regulators are shown in red arrow and negative regulators are shown in blue arrow. Factors that have been shown to directly bind *Gata3* gene are shown by a solid line, while other factors are shown with a dashed line. Factors that are regulated by GATA-3 protein positively (red arrow) or negatively (blue arrow) are shown. Genes that have been shown to be directly bound by GATA-3 are shown with a solid line, while genes for which evidence for direct regulation is lacking or questionable are shown with dashed lines. Factors shown in gray are suggested from correlative data.

fetal liver progenitors to Notch ligand for 3–5 days in OP9-DL1 co-cultures induced *Gata3* mRNA in DN1 cells, and this induction was attenuated in *Cbfb^{ns/-}* hypomorphic mutant hematopoietic cells (156). One interpretation of these data is that Notch signaling may drive *Gata3* gene expression through the RUNX/CBFβ complex during the earliest stages of T-cell development; a second is that development of *Gata3*-expressing cells may be impaired in *Cbfb^{ns/-}* hypomorphic mutant cells. We note that a direct effect of the RUNX complex on *Gata3* transcription has not been demonstrated to date.

The *E2a* gene encodes two alternatively spliced isoforms, E12 and E47. E47 cooperates with Notch to promote T-cell development from bone marrow progenitors (157) and *E2a*-deficient mice develop T-cell lymphomas that have a DN3-DP phenotype (158, 159). GFI1b, which is positively regulated by *E2A*, represses *Gata3* in an *E2a^{-/-}* lymphoma, and inhibition of *Gata3* by siRNA or by forced *Gfi1b* expression prevents the expansion and survival of *E2a^{-/-}* lymphoma cells (159). Repression of E protein activity in transgenic mice expressing *Id1* under the control of the *Lck* gene causes induction of *Gata3* mRNA in the CD4⁻CD8⁻CD44⁺CD25^{lo} cells that aberrantly accumulate in these mice (145). These observations suggest that *E2A* proteins might negatively regulate *Gata3* gene through *Gfi1b*. Since a role for endogenous *Gfi1b*, which is transiently induced at the DN3 stage, has not been reported and since the observation that forced transgenic expression of

Gfi1b under the control of the *Lck* promoter had little effect on DP T-cell development (160), the physiological significance of *Gata3* repression by *Gfi1b* will need to be examined much more extensively.

In DP thymocytes, GATA-3 is upregulated in response to TCR stimulation, and its expression level correlates with the strength of TCR signaling (56). The transcription factor MYB is required for T-cell development from the DN3 to DN4 stage, for survival and/or proliferation of DP cells, and for development of CD4 SP cells in the thymus (161, 162). Mice in which *Myb* was conditionally ablated at the DP and later stages (*Myb^{flox/flox};Tg^{Cd4cre}*) showed reduced expression of *Gata3* from DP to CD4 SP cells (163). Transgenic mice that express v-Myb, the oncogenic form of c-Myb, in T cells under the control of the CD2 promoter/LCR displayed about two-fold higher levels of *Gata3* mRNA in DP cells after positive selection (163). These data all implicate MYB in the regulation of *Gata3* during the DP to CD4 SP cell transition.

IL4/signal transducer and activator of transcription 6 (STAT6) and IL2/STAT5 pathways are required for Th2 CD4⁺ T-cell differentiation (reviewed in 135). Forced ectopic expression of STAT6 in Th1 cells by retroviral introduction of a STAT6-ER fusion protein followed by tamoxifen treatment induced *Gata3* and the Th2 cytokine genes *Il4* and *Il5* (164). Induction of *Gata3* under Th2-favoring conditions was abrogated in the absence of *Stat6* (165). Induction of the *Il4* gene by

GATA-3 (86) enhances *Gata3* activation by a positive-feedback loop. In contrast, retrovirally enforced expression of a constitutively active form of STAT5a did not induce *Gata3* expression but did trigger Th2 differentiation in the absence of IL4/STAT6 (166). Since this Th2 induction was abrogated in *Gata3*-deficient cells, endogenous GATA-3 appears to be required for STAT5-driven Th2 differentiation (98). GATA family proteins induce *Gata3* mRNA in *Stat6*-deficient peripheral T cells (167, 168). These data indicate that autoactivation supports *Gata3* activation in Th2 cells. Mice deficient in *Nfkb1* (encoding the p50 subunit of NF- κ B) show impaired production of Th2 cytokines after challenge with ovalbumin and reduced induction of GATA-3 (169). SAP and Viperin contribute to Th2 differentiation through NF- κ B (170, 171). These data indicate that NF- κ B-dependent *Gata3* induction is required for Th2 differentiation.

Inactivation of Notch signaling by germline deletion of *Rbpj* or transgenic expression of a dominant negative form of its cofactor, Mastermind-like 1, or by inactivation of *Notch1* and *Notch2* compromises Th2 responses (172–176). Retroviral transduction of the activated form of Notch in naive *Stat6*-deficient CD4⁺ T cells induced *Gata3* mRNA and GATA-3-dependent Th2 responses (175, 176). The activated form of Notch induces transcription of *Gata3* from the Ia promoter (177) but not from the Ib promoter, and ChIP experiments showed binding of RBPJ to the *Gata3* Ia promoter (175, 176). These data indicate that the Notch signaling pathway induces Th2 responses at least in part by directly activating the *Gata3* gene. Although Notch signaling activates the Ia promoter, it has also been shown that Ib transcripts are 100-fold more abundant than Ia transcripts during Th2 activation (178). Activation of *Gata3* by the Notch signaling pathway is also observed at earlier stages of T-cell development (156), and loss of either factor resulted in impaired ETP development (27, 85). Furthermore, both Notch signaling and GATA-3 play important roles that are independent of pre-TCR complex formation required to pass the β -selection checkpoint (57, 179). Although direct regulation of the *Gata3* gene by Notch effectors has not been shown except during peripheral stages, these data suggest that collaborative roles for Notch and GATA-3 are required repeatedly for T-cell development from the beginning to the end of T-cell development.

CD4⁺CD25⁻ T cells isolated from mice ablated for the *Tcf7* gene (encoding transcription factor TCF1) or from transgenic mice expressing ICAT, a specific inhibitor of the interaction of TCF1 and its cofactor β -catenin, showed impaired Th2 differentiation and reduced expression of *Gata3* mRNA transcribed from the *Gata3* Ib promoter compared to control cells, while

transgenic mice expressing β -catenin showed the opposite response (178). ChIP experiments revealed binding of TCF1 and β -catenin to the *Gata3* Ib promoter in stimulated CD4⁺ T cells (178). These data indicate that TCF1-dependent *Gata3* activation is required for Th2 differentiation.

Mice ablated for *Bhlhe41* (encoding transcription factor DEC2) showed impaired Th2 differentiation, and the CD4⁺ T cells isolated from those mutant mice had reduced GATA-3 mRNA in Th0 or Th2 cultures (149). Retrovirally directed expression of DEC2 greatly induced *Gata3* transcription under Th2- or Th17-polarizing conditions, but it was only slightly induced under Th1-polarizing conditions (149). ChIP experiments using cells isolated from transgenic mice expressing FLAG-tagged DEC2 under the control of the CD2 promoter/LCR showed DEC2 binding to *Gata3* (149). These data indicate that DEC2-dependent *Gata3* induction is required for Th2 differentiation.

The transcription factor TBET, a master regulator of Th1 cell differentiation, represses the *Gata3* gene (180). In addition to exerting transcriptional control by *Gata3* repression, TBET may bind directly to GATA-3 protein and thereby sequester it from binding to target DNA sites (181). RUNX1, which promotes Th1 differentiation, represses the *Gata3* gene (182). Additionally, RUNX3, which cooperates with TBET to promote Th1 differentiation, interacts with GATA-3 and suppresses its transcriptional activity (183). In contrast to its repressive effect at the Th1 stage, the RUNX/CBF β complex may actually function as an activator of *Gata3* at early stages (156). Since both GATA-3 and RUNX complexes play important roles at multiple stages (early, late, and peripheral) of T lymphopoiesis, it will be intriguing to elucidate the similarities and differences of their possible interplay at each stage. TGF β supports Th17 differentiation by repressing *Gata3* and *Stat4* (184). The aryl hydrocarbon receptor (AhR) and its agonist M50354 also seem to skew the Th1/Th2 balance toward Th1 dominance by repressing *Gata3* (185).

These upstream factors have all been implicated to exert direct control over *Gata3* transcription through the promoter or putative enhancers of the *Gata3* gene (Table 3), or indirectly by controlling other direct regulators or by modifying chromatin status within the locus (Fig. 2). We have found that *Gata3* is expressed in up to two dozen discrete tissue types, and each expression pattern appears to be driven by a discrete, individual tissue-specific enhancer element (103, 106, 186–188, our unpublished data). To initially localize many of these regulatory elements, mice harboring reporter transgenes containing as little as 300 bp to mice bearing as much as 662 kbp of *Gata3* genomic DNA have been examined in trans-

Table 3. Binding sites of proposed upstream transcription factors

Transcription factor	<i>Gata3</i> regulation	Recovered region by ChIP	ChIP	Reference
MYB	CD4 SP	382–618 bps upstream of I β	Total thymocytes	(163)
RBPJ	Th2	291–398 bps upstream of I α	CD4 ⁺ T	(175)
RBPJ	Th2	154–310 bps upstream of I α	Stimulated CD4 ⁺ T	(176)
TCF1	Th2	550–650 bps upstream of I β	Stimulated CD4 ⁺ T	(178)
DEC2	Th2	438–1072 bps downstream of I β (1st intron and 2nd exon)	Naïve CD4 ⁺ T	(149)

fection and transgenic reporter assays. These studies identified regulatory elements for *Gata3* that specify its expression in the 1st and 2nd branchial arches, the genital tubercle/cloaca, in mammary epithelial cells, hair follicles, vestibulocochlear ganglia, the central nervous system (three separate enhancers), the eye, the ectoplacental cone/placenta, the thyroid gland, the mesonephric and metanephric tubules of the embryonic kidney, the semicircular canals of the inner ear and primordia specifying the endocardial cushions. Perhaps surprisingly, these studies also indicated that the regulatory elements that control *Gata3* expression in the sympathetic nervous system probably lie beyond 451 kbp 5' or 211 kbp 3' (as defined by one transgenic YAC; the genome sequence-revised endpoints) with respect to the GATA-3 translational start site. However, since all these studies were performed between days 10.5 and 14.5 of embryogenesis, the element specifying *Gata3* expression in mature T cells was not examined (since the first DP cells develop at around e16.5) (189). We have recently found that this same 662 kbp *Gata3* YAC does not harbor sufficient sequence information to direct reporter gene expression in adult thymocytes, but intriguingly we have now identified multiple overlapping bacterial artificial chromosomes (BACs) that can activate a *Gata3*-directed reporter gene in T cells *in vivo* (our unpublished observations). The developmental and temporal specificity of this regulatory sequence(s) may reveal profound new insights into the regulation of *Gata3*, and therefore indirectly into the molecular mechanisms controlling T-cell development.

Conclusions

We have reviewed here the requirements for and known functions of GATA-3 in T-lineage cells from the earliest T-cell pro-

genitors in the thymus all the way to peripheral CD4⁺ Th2 cells. Growing evidence has begun to reveal the hierarchical positioning of GATA-3 and other molecules in these important developmental processes, especially in peripheral Th2 cell activation. In contrast, only a handful of factors have been demonstrated to interact with GATA-3 during T-cell development in the thymus. The development of next-generation sequencing technology has made it possible to comprehensively identify direct targets of a transcription factor through whole genome ChIP sequencing and whole genome mRNA sequencing. Subsequent molecular biological confirmation of the functional importance of these genomic indications will be essential to understand the transcriptional hierarchy mediated by and through GATA-3. To these ends, the *Gata3* hypomorphic mutant as well as the *Gata3* null and conditional alleles should prove to be indispensable tools. As described above, a skeletal network description for immature T-cell development has been published and should serve as an outstanding framework for probing aspects of transcriptional regulation during immature T-cell development (145). Although much more data will be required to elaborate the influences and nuances in this network that will produce a precisely refined picture of the process as a whole, overwhelming evidence supports the hypothesis that GATA-3 must play a central role at multiple stages of T-cell development, and therefore, it must be a fundamental contributor to many steps in the overall framework from the birth to the death of T cells. It will be fascinating both to test mechanistically how the network is maintained as well as to learn what (known and currently unknown) molecules participate in the establishment of T-cell development.

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