The Interplay between Transcription Factors and Epigenetic Modifications in Th2 Cells

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Abstract

Functionally polarized CD4 T helper (Th) cells, such as Th1, Th2, and Th17 cells, are essential for the regulation of acquired immunity. Differentiation of naïve CD4 T cells into Th2 cells is characterized by chromatin remodeling and the induced expression of a set of Th2-specific genes, which include Th2 cytokine genes. In the first stage of this differentiation, a Th2-skewing cytokine environment, especially IL-4, induces STAT6 activation. Activated STAT6 increases the expression of GATA3, a master regulator of Th2 cell differentiation, via direct binding to the Gata3 gene locus. This transcriptional induction of Gata3 mRNA during Th2 cell differentiation is accompanied by dynamic changes in the binding patterns of two epigenetic modification proteins such as Polycomb and Trithorax complexes. Consequently, expressed GATA3 epigenetically modifies and upregulates Th2-specific genes to establish Th2 cell identity. This identity is maintained by high-level expression of the Gata3 gene controlled by Menin, which is a member of the Trithorax proteins, after cycles of cultivation in vitro and a long-term resting state in vivo. Thus, the Menin-GATA3 axis handles the Th2-specific gene regulatory network.

Keywords: Th2, GATA3, STAT6, Menin

1. Introduction

Naïve CD4-positive (CD4+) T cells can differentiate into several effector T cell subsets, mainly known as Th1, Th2, and Th17 cells [1]. Th1 cells perform the crucial function of protecting against viruses and intracellular pathogens. Th17 cells similarly work against extracellular bacteria or fungi. Th2 cells are required for the removal of extracellular parasites. Each effector subset exerts its protective functions through the secretion of unique cytokines. Th1 cells mainly produce IFN-γ, which activates macrophages and CD8 T cells. Th17 cells secrete IL-17A, which
propagates cascades of events that lead to neutrophil recruitment, inflammation, and host defense [2]. Th2 cells activate B cells to induce immunoglobulin class switching through IL-4, and enhance mucus production from epithelial cells by IL-13. In addition, Th2 cells recruit eosinophils to induce an inflammatory response through IL-5. However, the responses caused by these subsets are sometimes excessive and result in immunological diseases. For example, an excess amount of Th2 cytokines is known to induce allergic disease, such as asthma [3].

Each subset-specific cytokine enhances differentiation toward the corresponding Th subset, and environmental cytokines decide the differentiation fate of CD4 T cells. For example, IL-12-induced STAT4 activation in Th1 cells and IL-4-induced STAT6 activation in Th2 cells are essential for their respective differentiation [4, 5]. These STAT signals are commonly used for CD4 T cell differentiation into each subset and induce the upregulation of master transcription factors, T-bet in Th1 and GATA3 in Th2 [6, 7]. The master transcription factors directly bind to DNA and regulate the expression of each subset-specific gene, causing epigenetic modification of the DNA, which stabilizes the differentiation program. Due to this epigenetic modification, fully differentiated effector T cells are rarely converted to other Th subsets and are able to maintain their identity during the transition from effector to memory cells.

The Th2 master transcription factor GATA3 collaborates with the epigenetic regulator Menin to induce and stabilize the complex gene regulatory network. Th2-specific genes, which have been identified by gene expression profiling [8, 9], participate in this regulatory network and are controlled by neither, either or both GATA3 and Menin [10]. In fact, GATA3 or Menin deletion results in the loss of Th2 identity [10, 11]. Clarifying the interplay between the transcription factors and epigenetic modifiers is required to comprehend the Th2 cell biology and to identify new therapeutic targets for Th2-mediated immunological diseases [3].

2. STAT6 and GATA3: important transcription factors for Th2 cells

2.1. STAT6 is activated by IL-4 signaling

The most essential pathway promoting the Th2 fate is the IL-4 signaling cascade, followed by activating the transcription factor STAT6 [12–14]. When IL-4 is recognized by its receptor (type-I IL-4R), which consists of IL-4 receptor alpha chain (IL-4Rα) and a common gamma chain (γc), IL-4 can transmit a signal into a cell. Binding of IL-4 induces dimerization of IL-4Rα and γc, resulting in the phosphorylation of tyrosine residues within the intracellular portion of IL-4Rα by Janus Kinases. This phosphorylated intracellular portion of IL-4Rα recruits and phosphorylates signal transducer and activator of transcription (STAT)6, which then forms a dimer and translocates into the nucleus where the dimerized STAT6 regulates the expression of IL-4 target genes. STAT6 recognizes the DNA sequence TTCNNNGAA, whereas other STAT family proteins prefer the DNA sequence TTCNNNGAA [15].

Like other STAT proteins, a major role of STAT6 is to activate the expression of its target genes, which is how it received its name (“signal transducer and activator of transcription”). The best-known target gene of STAT6 is the Gata3 gene, and the detailed mechanisms underlying the STAT6-dependent regulation of the Gata3 gene are described in Section 4. However, some studies have
reported that STAT6 also exerts an inhibitory function by occupying overlapping binding sites of other transcription factors and blocking their binding [16, 17]. It is now well known that STAT-mediated repression is important for the lineage commitment of Th subsets [18]. For example, STAT6 binds to the genomic loci of Th1-associated genes and inhibits their expression, and STAT4, a key transcription factor of Th1, acts on Th2-associated genes in a similar way [19].

It has been proposed that the IL-4/STAT6 cascade is necessary for the Th2 phenotype. This fact is also demonstrated by a series of knockout studies. In these studies, IL-4 deficient mice showed impaired Th2 responses, attributed to a reduced Th2 effector cytokine production, loss of IgE class switching, and reduced eosinophilia upon infection with *Nippostrongylus brasiliensis* [20]. A similar but more significant phenotype is observed in STAT6 knockout mice. In addition, STAT6 appears to be highly specific to Th2 functions, as the phenotype of STAT6-deficient mice is largely related to the loss of the Th2 cell function, and deficient mice show normal development with ordinary numbers of T cells [21, 22]. Other STAT signaling cascades are also involved in Th2 polarization. STAT5A and STAT3, which are activated by IL-2 [23] and IL-6 [24], respectively, are also reported to induce the Th2 phenotype. However, STAT5 and STAT3 are activated not only in Th2 but also in other CD4+ T cell subsets. Therefore, only STAT6 exclusively promotes Th2 differentiation.

2.2. GATA3 plays roles in various tissues as well as the immune system

The GATA family proteins (GATA1–6) are conserved transcription factors that contain one or two C2-C2-type zinc-finger motif that recognize the consensus DNA sequence WGATAR [25–27]. Each member of the GATA family has different expression patterns in the body and can be grouped into hematopoietic factors (GATA1–3) and endodermal factors (GATA4–6). Among hematopoietic cells, immune cells, particularly developing and mature T cells, natural killer (NK) cells, and CD1-restricted NKT cells, mainly express GATA-binding protein 3 (GATA3) [6, 28, 29]. Mature mast cells express GATA1 and GATA2 but not GATA3 [30]. Outside of the immune system, GATA3 is also expressed in many embryonic and adult tissues, including the adrenal glands, kidneys, central nervous system, inner ear, hair follicles and skin, and breast tissue [27].

In the immune system, GATA3 is predominantly expressed in T lymphocytes and is essential for the development of CD4 single-positive (SP) cells in the thymus [31–33]. GATA3 exerts an important function at the β-selection checkpoint, which is involved in the CD4 versus CD8 lineage choice in the thymus [34]. It is continuously expressed in peripheral naïve CD4 T cells at a basal level, where the activation of STAT6 induced by the IL-4/IL-4 receptor signaling pathway upregulates *Gata3* mRNA expression during Th2 cell differentiation [35]. GATA3 is thought to be necessary as the master regulator of Th2 differentiation [6, 7], since enforced GATA3 expression induces Th2 differentiation even when the cells are cultured under Th1-skewing conditions [35]. Enforced expression of GATA3 has also been reported to endogenously upregulate GATA3 expression [36]. In addition, the amount of GATA3 protein in Th2 cells is regulated by various posttranscriptional mechanisms [37–39]. Furthermore, high-level expression of GATA3 is essential for the production of large amounts of Th2 cytokines in established Th2 cells [11, 40–42]. The detailed mechanisms underlying the GATA3-dependent regulation of its target genes are described in Section 5.
3. Polycomb and Trithorax proteins: fundamental epigenetic regulators for cell differentiation

3.1. Polycomb and Trithorax proteins epigenetically modify chromatin in a different way

Huge numbers of genes involved in epigenetic regulation have been identified. Many of them encode histone-modifying enzymatic proteins and their interaction partners. Among them, members of the Polycomb group (PcG) and Trithorax group (TrxG) complexes have been recognized as key epigenetic regulators [3, 43–46]. PcG and TrxG proteins were originally identified in Drosophila; however, they also play essential roles in controlling mammalian gene expression in various normal and tumor tissues. It has long been thought that PcG and TrxG proteins antagonize each other for turning target gene expression off or on, respectively. PcG proteins mediate gene silencing by controlling the repressive histone mark H3K27me3 (trimethylated histone H3 lysine 27), whereas TrxG proteins mediate gene activation by modifying the permissive histone mark H3K4me3. Both histone-modifying complexes are often found to regulate the same genes at different stages of development [47]. In addition, emerging evidence shows that PcG and TrxG proteins participate in complex regulatory mechanisms in mammalian tissues [48].

PcG complexes are classified into two canonical types such as Polycomb repressive complex 1 (PRC1) and PRC2. Both of them are involved in transcriptional repression. A sequential recruiting mechanism is proposed for the binding of PRC2 and PRC1 to genomic DNA. First, enhancer of zeste (EZH), the enzymatically active subunit of PRC2, methylates H3K27. Next, the PRC1 complex recognizes trimethylated H3K27, resulting in its co-localization with PRC2. In addition, the ring finger protein 1 (RING1), a subunit of PRC1, has a ubiquitin ligase activity for histone H2AK119 [49]. In CD4+ T cells, Ezh2 appeared to directly bind and facilitate the correct expression of the Gata3 gene during differentiation into effector Th2 cells [50, 51]. In our previous study, Ezh2 bound much more strongly to transcription factor genes, including the Gata3 gene, than to the cytokine or cytokine receptor genes. Genome-wide, in the genes encoding transcription factors, the Ezh2 binding levels appear to be higher in non-expressed genes than in expressed genes [52].

In contrast, mixed lineage leukemia (MLL) family proteins, which are major subunits of the TrxG complex, have H3K4 methyltransferase activity that induces a change in the chromatin structure to a form permissive for transcription. In mammals, six H3K4 methylases (MLL1–4, SET1A, and SET1B) have been discovered [53]. The H3K4 methylase complexes containing MLL1 or MLL2 are associated with a unique subunit named Menin (encoded by the Men1 gene in mice). A mutation of MEN1 has been found in patients with multiple endocrine neoplasia type 1 (MEN1) syndrome [54, 55]. Menin can act as a tumor suppressor and is required for TrxG complex binding to DNA [53]. Menin is also indicated to have essential roles in the immune system, as Menin has been shown to be important for the Th2 cell function both in mice and humans [51, 56]. The MLL3- or MLL4-containing complex associates with the H3K27 demethylase UTX (encoded by the Kdm6a gene in mice) and induces demethylation. H3K4 trimethylation appears to be mediated by these MLL-associated complexes in a gene-specific manner. The SET1A- or SET1B-containing complexes have the unique WD repeat-containing 82 (WDR82). TrxG proteins activate target gene expression and/or keep them active, indicating
that these proteins are associated with more than simple gene activation [53]. TrxG proteins have more diverse binding molecules than PcG proteins with which they form complexes.

### 3.2. Spatial interplay between Polycomb and Trithorax complexes

Although many studies have been performed on the nature of PcG proteins and TrxG proteins individually, few have successfully defined how transcriptional counter-regulation is organized by the PcG and TrxG complexes. One pioneering work demonstrated the dynamic transformations of histone modifications during T cell development [57]. In addition, in our previous study, we successfully analyzed how the global signature of PcG and TrxG co-occupied genes changed during the developmental process. This study showed that a binding pattern in which Ezh2 binds upstream and Menin binds downstream of the transcription start site was frequently found at highly expressed genes, and a binding pattern in which Ezh2 and Menin bind to opposite positions was frequently found at low-expressed genes in T lymphocytes. Interestingly, genes showing a binding pattern in which Ezh2 and Menin occupied the same position displayed greatly enhanced sensitivity to Ezh2 deletion [3, 58].

### 4. STAT6 induces dynamic changes in epigenetic states at the Gata3 gene locus

#### 4.1. The Gata3 gene is epigenetically regulated during Th2 cell differentiation

Epigenetic changes at the Gata3 gene locus in T cells are essential for the acquisition and maintenance of the Th2 cell identity [3, 51, 59]. During Th2 cell differentiation, PcG and TrxG proteins dynamically change their binding patterns at the Gata3 gene locus. In addition, these epigenetic changes result in GATA3 protein upregulation that consequently induces chromatin remodeling at the Th2 cytokine gene loci, including Il4, Il5, and Il13 [51, 59]. The Gata3 gene is known to have distal and proximal promoters. Both basal transcription in naïve CD4 T cells and induced transcription in differentiated Th2 cells are controlled by the proximal promoter [51, 60]. In naïve CD4 T cells, PcG complexes bind upstream and TrxG complexes bind downstream of the Gata3 proximal promoter [51]. During Th2 cell differentiation, PcG proteins dissociate upstream of the Gata3 proximal promoter, and the binding of TrxG proteins spreads into this region. Consequently, rapid alterations in the binding patterns of PcG and TrxG proteins are observed in the region between the Gata3 distal and proximal promoters in this period. Histone modification patterns basically exhibit the same behavior; H3K27me3 levels are decreased at the upstream region of the Gata3 proximal promoter, and H3K4me3 spreads into this region. In contrast, changes in DNA methylation pattern are only observed at exon 2, in which DNA is methylated in naïve CD4 T cells and demethylated in Th2 cells [61]. At present, the mechanism underlying this demethylation process remains unclear.

#### 4.2. STAT6 directly modifies epigenetic states at the Gata3 gene locus

We identified two functional STAT6 binding sites within the intronic regions of the Gata3 gene locus [51]. A chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq)
analysis also identified one STAT6 binding site at the same region [62]. In the absence of STAT6, displacement of PcG by TrxG is not observed. These results indicate that STAT6 directly binds to the Gata3 gene locus and induces PcG/TrxG displacement, although the precise mechanism is still unclear. A study of human Th2 cells indicated that STAT6 binding was hardly detected at the GATA3 gene locus, although STAT6 knockdown was effective for reducing the GATA3 expression [18]. Interestingly, our ChIP-seq analysis detected one GATA3 binding peak close to one of the STAT6 binding sites at the Gata3 gene locus [8, 51] and one of the strong peaks on the assay for transposase-accessible chromatin sequencing (ATAC-seq) [63]. This GATA3 binding site may be important for cis-regulation via GATA3-dependent auto activation of the Gata3 gene [36]. Although STAT6 induces TrxG spreading into the promoter region, the T cell-specific deletion of Menin, a component of the TrxG complex, does not affect Th2 cell differentiation. This suggests that the induction of high-level expression of Gata3 (i.e. the acquisition of the Th2 cell identity) is dependent on STAT6 and not the Menin/TrxG complex [51]. However, the maintenance of the Gata3 expression is dependent on the Menin/TrxG complex and independent of IL-4 and STAT6 in Th2 cells. A similar molecular mechanism was found to underlie the Gata3 expression in vivo [10, 64]. In human memory Th2 cells, MLL and Menin form a core transcriptional complex and regulate the GATA3 expression [65]. Therefore, TrxG proteins represent an essential mechanism underlying transcriptional maintenance in the memory Th2 cell response [3].

4.3. PRC2 components prevent hyperactivation of the Gata3 gene

In contrast to TrxG proteins, PcG proteins are proposed to maintain their Gata3 expression at an appropriate level in CD4 T cells [3]. T cell-specific deletion of Ezh2 enhances the sensitivity of IL-4 and results in Gata3 upregulation and hyper-production of Th2 cytokines [50]. A ChIP-seq analysis revealed that the Ezh2 binding levels were high at the Gata3 gene locus but very low at the Th2 cytokine gene loci, indicating that Ezh2 controls the Th2 cytokine expression via direct binding to the Gata3 gene locus. However, measurable levels of H3K27me3 were detected at the Il4 and Il13 genes loci, and direct regulation of H3K27me3 by Ezh2 at these genes has also been proposed as important for transcriptional silencing in Th1 cells [66]. In contrast, SUV39H1-dependent H3K9me3 has been found to maintain the silencing of Th1 cell-related genes in Th2 cells [67].

5. GATA3-dependent epigenetic and transcriptional regulation in the Th2 cytokine gene loci

5.1. Chromatin remodeling induced by GATA3 at the Th2 cytokine gene loci

Induction of changes in histone modifications has been reported at the Il4, Il5, and Il13 gene loci (so-called the Th2 cytokine gene loci) during Th2 differentiation [12, 59, 68]. Particularly, histone H3K4 methylation and H3K9 acetylation play an important role in forming the open chromatin structure. Thus, the regions that acquire these histone modifications become accessible to transcription factors and are frequently associated with DNase I hypersensitive (HS) sites. Chromatin remodeling at the Th2 cytokine gene loci is necessary for the efficient expression of IL-4, IL-5, and
IL-13 in Th2 cells, and GATA3 has been proposed to regulate chromatin remodeling at these genes. Notably, the H3K9 acetylation levels are higher around the GATA3 binding sites at the Th2 cytokine gene loci than the regions without GATA3 binding [8]. However, genome-wide surveys on GATA3 binding and histone modifications suggest that GATA3 binding do not perfectly coincide with changes in permissive histone modifications, which correlate highly with the states of transcription [62, 69]. In fact, some studies suggest that GATA3 acts not only as an activator but also as a repressor in both Th1 and Th2 cells [8]. Although GATA3 is recognized as a master regulator of Th2 cell differentiation, the transcription of many Th2-specific genes is not regulated by GATA3 itself; therefore, GATA3 is not the only essential factor for Th2 differentiation.

5.2. Interaction between GATA3 and regulatory elements

It has been reported that GATA3 interacts with some regulatory elements at Th2 cytokine gene loci, including conserved non-coding sequence (CNS)-1, HSVa, the conserved GATA response element (CGRE), and HSII in intron 2 of the Il4 gene [12, 68, 70–74]. CNS-1 is located at the intergenic region between the Il4 and Il13 genes and was originally described as Th2-specific HS sites (HSS1 and HSS2) [75, 76]. To characterize the function of CNS-1, mice lacking this genomic region was generated [77]. Genetic deletion of the CNS-1 region resulted in a reduction of Th2 cells producing IL-4, IL-5, and IL-13. In this mutant mouse, IL-4 production in vivo was also abrogated [77]. However, CNS-1-deficiency had no effect on IL-4 production in bone marrow-derived mast cells [78]. This is consistent with the observation of no HS sites in CNS-1 of mast cells. Although an electrophoresis mobility shift assay showed that GATA3 binds to HSS2 in vitro [70, 71], two independent genome-wide GATA3 ChIP-seq data analyses failed to detect significant GATA3 binding peak in the CNS-1 region (Figure 1) [8, 79].

![Figure 1](The Interplay between Transcription Factors and Epigenetic Modifications in Th2 Cells http://dx.doi.org/10.5772/intechopen.73027)
Taken together with the fact that histone acetylation levels are increased with progressive DNA demethylation in the CNS-1 region [61, 80], this region may recruit other critical transcription factors that induce epigenetic modifications and promote IL-4 production in Th2 cells.

HSVα is a TCR re-stimulation-dependent HS site, whose DNase I hypersensitiveness is induced in Th2 cells upon stimulation [72]. HSVα is located 5 kbp downstream of the 3’ end of the Il4 coding region. Th2 cells generated from the mice in which the genomic region containing both HSVα and HSV (CNS-2) has been deleted display a reduced IL-4 production [81]. Another study reported on the phenotypes of mice with genomes containing the specific deletion of the CNS-2 region [82]. Mice lacking CNS-2 display marked defects in Th2 humoral immune responses. However, the effector Th2 cells involved in tissue responses were not likely to be dependent on CNS-2. In this region, increased histone acetylation levels are observed. In contrast, changes in DNA methylation state are not induced, as DNA is demethylated even in naïve CD4 T cells [80]. By using a conventional ChIP technique, both GATA3 and nuclear factor of activated T cells 1 (NFAT1) have been shown to bind to HSVα in Th2 cells [72]. We and others have performed a GATA3 ChIP-seq analysis and detected GATA3 binding peaks at the HSVα [8, 79], implying that HSVα functions as an important regulatory element through which GATA3 and NFAT1 collaborate to induce IL-4 production in stimulated Th2 cells.

As we reported in 2002, CGRE was originally identified as a region with a 71-bp sequence located 1.6 kbp upstream of the Il13 gene [73]. The location of CGRE corresponds approximately to the site of HSI. CGRE contains four putative GATA-binding sequences conserved across species [73]. Strong signals of GATA3 binding have been detected by both conventional ChIP assay and ChIP-seq analyses at the CGRE [8, 9, 79]. Interestingly, CGRE is also located at the 5’ edge of the region of histone hyperacetylation, suggesting that GATA3 binds to the CGRE and induces histone acetylation toward the 3’ region of the Il13 gene. Indeed, GATA3 associates with RNA polymerase II and CBP/p300, which contain histone acetyltransferase activity at this region [73]. In addition, CGRE is located at the 5’ edge of the accessible DNA region detected by ATAC-seq [63]. Thus, the CGRE region may play an important role in Il13 transcription and in chromatin remodeling at the Il13 locus. Notably, the Th2 cells generated from CGRE-deficient mice exhibit diminished IL-13 but not IL-4 or IL-5 production [74].

Among several GATA3 binding sites found in the Il4 gene locus, the strongest GATA3 binding signal was detected at the HSII site located in intron 2 of the Il4 gene [8]. This region also contains binding sites for STAT5, which has been reported to be important for the maintenance of DNA accessibility of this region in Th2 cells [23, 83]. Correspondingly, a strong ATAC-seq peak was detected at this region in Th2 cells [63]. Recently, a group reported that genetic deletion of HSII resulted in a reduction in IL-4 but not IL-13 production, implying its role in regulating IL-4 production [74]. In addition to histone hyperacetylation induced in this region, H3K4me3 was strongly induced at HSII in Th2 but not Th1 cells [84], suggesting that GATA3 may work together with STAT5 and remodel the chromatin structure at this region. Parallel to changes in histone modifications, progressive DNA demethylation was observed across the Il4 gene locus. In naïve CD4 T cells, only the promoter region of the Il4 gene is demethylated, and DNA demethylation extends into the Il4 gene body during Th2 cell differentiation [80].
5.3. GATA3-dependent transcriptional regulation of Th2 signature genes

In addition to regulating chromatin remodeling, GATA3 may induce *Il5* and *Il13* transcription by directly binding to the promoters of these cytokine genes upon TCR re-stimulation [7, 85–87]. In fact, *Gata3* siRNA knockdown just before TCR re-stimulation resulted in reduced expression of *Il5* and *Il13* in established Th2 cells (Figure 2). The role of GATA3 in *Il5* and *Il13* transcription was also reported using genetic deletion of the *Gata3* gene. While GATA3 deletion during Th2 differentiation abolished the expression of all Th2 cytokines, GATA3 deletion in established Th2

![Graph](image-url)

**Figure 2.** The effects of GATA3 knockdown on the Th2-specific genes in effector and memory Th2 cells. The effects of GATA3 knockdown on effector Th2 (upper) and memory Th2 (lower) were determined with qRT-PCR (originally published in *PLoS ONE*. Sasaki et al. [9]). The relative expression (GATA3/control siRNA) is rank-ordered and shown as a percentage. The genes indicated in the red bar showed increased GATA3 dependency in memory Th2 cells, while those in blue showed decreased GATA3 dependency.
cells strongly influenced the expression of both IL-5 and IL-13 and induced only a modest reduction in IL-4 production [42]. GATA3 is also crucial for the expression of the Th2 cytokine genes in memory Th2 cells, as Gata3 siRNA knockdown reduces the transcription of those genes [9]. Furthermore, GATA3 is involved in the transcriptional regulation of other Th2 signature genes in both effector and memory Th2 cells (Figure 2). Approximately half of the Th2-specific genes (16 out of 31) showed a significant reduction in their expression in effector Th2 cells (F2r, Mapk12, Il1r2, Il4, Tanc2, Ptgir, Nfil3, Rnf128, Asb2, Tnntc2, Ccnj1, Gata3, Cyp11a1, Il13, Ccr8, and Il5) by GATA3 knockdown. In contrast, only the Tube1 gene showed a significant increase in its expression. These results suggest that a major role of GATA3 is the activation of its target gene transcription.

Interestingly, changes in GATA3 dependency are observed during transition from effector to memory cells. In a previous study [9], we compared the GATA3 dependency in Th2-specific genes between effector Th2 cells and in vivo-generated memory Th2 cells by Gata3 siRNA knockdown. GATA3 dependency increased by more than twofold in the Epas1 and Il24 genes in memory Th2 cells compared to the effector Th2 cells. In addition, for the Tube1, Rnf128, Ccr8, and Il5 genes, the GATA3 dependency decreased by more than twofold. These results indicate that each Th2-specific gene differentially changes its dependency on GATA3 during maturation to memory Th2 cells from effector Th2 cells. The changes in GATA3 dependency, however, do not correlate with dependency itself. For example, Il5 is a gene with high dependency on GATA3 that shows a decreased dependency in memory Th2 cells. Taken together, these findings indicate that GATA3 is important for maintaining the transcriptional signatures in established Th2 cells.

6. A gene regulatory network in fully developed Th2 cells: the interplay between GATA3 and Menin, a component of the Trithorax complex

As described in Section 4.2, although Menin deficiency had little effect on the ordinary induction of Th2 differentiation, ‘Th2 cells’ lost their Th2 identity after several cycles of cultivation in the absence of Menin. Our study also revealed that Menin directly bound and epigenetically regulated the Gata3 gene, suggesting that constant expression of Menin and its binding to the Gata3 locus is necessary for the maintenance of the Th2 identity. Similar results were obtained with in vivo-generated memory Th2 cells, indicating that Menin maintains the memory Th2 cell function during the long-term resting phase. Indeed, Menin-deficient memory Th2 cells show an impaired ability to recruit eosinophils to the lung, causing the attenuation of airway inflammation induced by memory Th2 cells [52]. Since Th2 cells derived from Menin-deficient mice have defects in both Menin and GATA3 expression, whether the lack of Menin, decreased expression of GATA3, or both are responsible for the dysregulation of the Th2-specific gene expression in Menin-deficient cells remains unclear. In a recent study [52], we addressed this point using differentiated Th2 cells with two additional cycles of cultivation (Th2-3rd cells). Consequently, the gene expression profiles under three conditions (i.e. genetic deletion of Menin, Gata3 siRNA treatment, and retroviral gene transduction of hGATA3) were used to classify the Th2-specific genes into four groups (Figure 3). Asb2, Ccr8, Gzma, Il4, Il5, Il13, Il24, Mapk12, Tanc2, and Tube1 were assigned to Group 1, being
controlled by both GATA3 and Menin. Interestingly, only Gzma was negatively regulated by Menin, while the other nine genes were positively regulated. Although Gata3 siRNA treatment downregulated the Gzma expression, the forced expression of hGATA3 also reduced the Gzma expression for some unknown reason. Seven genes (Crem, Cyp11a1, F2r, Nfil3, Ptgir, Rnf128, and Tnmtc2) were found to be positively controlled by GATA3 and not affected by Menin deficiency (Group 2). Group 3 consisted of Spry2 and S100a, which were found to be controlled in a Menin-dependent and GATA3-independent manner. For the other 11 genes (Ccnj1, Dusp4, Ecm1, Epas1, Grtp1, Il1r2, Itgb3, Jdp2, Penk, Plcd1, and Tnfrsf8), neither Gata3 knockdown nor Menin deficiency had a significant effect on the gene expression (Group 4).

In our ChIP-seq analysis, the direct binding of Menin was observed in most of the 31 Th2-specific genes, except for Asb2, Mapk12, Ecm1, Grtp1, and Plcd1. Nine of the Menin target genes (Ccr8, Gata3, Il4, Il5, Il13, Il24, S100a1, Tanc2, and Tube1) were positively regulated by Menin, whereas two targets (Gzma and Spry2) were negatively regulated. No significant effect of Menin deficiency was observed on the other 15 targets (Ccnj1, Crem, Cyp11a1, Dusp4, Epas1, F2r, Il1r2, Itgb3, Jdp2, Nfil3, Ptgir, Penk, Rnf128, Tnmtc2, and Tnfrsf8). Several questions remain to be addressed regarding this regulatory network: Are any other factors involved? What recruits Menin to these gene loci? Why does Menin exert a suppressive effect on some target genes? (Table 1).

**Figure 3.** Th2-specific gene regulatory network. The regulatory network formed by Menin- and Th2-specific genes, including GATA3 (originally published in *The Journal of Immunology*, Onodera et al. [10]). Group 1 contains genes that are controlled by both GATA3 and Menin. Genes in Groups 2 and 3 are controlled by either GATA3 or Menin, respectively. Group 4 includes genes that are affected by neither GATA3 knockdown nor Menin knockout. Red arrows indicate the regulatory interactions that activate the target gene expression, whereas blue lines indicate the suppressive effects for targets.
| RefSeq ID | Gene symbol | Group | GO term (function, process, or component) |
|-----------|-------------|-------|------------------------------------------|
| NM_008355 | Il13        | 1     | Cytokine activity                        |
| NM_010558 | Il5         | 1     | Cytokine activity [88]                   |
| NM_023049 | Asb2        | 1     | Contributes to ubiquitin protein ligase activity [89] |
| NM_028006 | Tube1       | 1     | GTPase activity                          |
| NM_013871 | Mapk12      | 1     | MAP kinase activity                      |
| NM_010370 | Gzma        | 1     | Serine-type peptidase activity [90]      |
| NM_053095 | Il24        | 1     | Cytokine activity [91]                   |
| NM_007720 | Ccr8        | 1     | C-C chemokine receptor activity [92]     |
| NM_021283 | Il4         | 1     | Cytokine activity [93]                   |
| NM_181071 | Tanc2       | 1     | In utero embryonic development [94]      |
| NM_017373 | Nfili3      | 2     | RNA polymerase II core promoter sequence-specific DNA binding [95] |
| NM_013498 | Crem        | 2     | Core promoter sequence-specific DNA binding [96] |
| NM_008967 | Ptgir       | 2     | G-protein coupled receptor activity      |
| NM_010169 | F2r         | 2     | G-protein alpha-/beta-subunit binding [97] |
| NM_019779 | Cyp11a1     | 2     | Cholesterol monooxygenase (side-chain-cleaving) activity [98] |
| NM_177368 | Tmtc2       | 2     | Calcium ion homeostasis                  |
| NM_023270 | Rnf128      | 2     | Ubiquitin protein ligase activity [99]  |
| NM_011309 | S100a1      | 3     | Protein binding [100]                    |
| NM_011897 | Spry2       | 3     | Negative regulation of ERK1 and ERK2 cascade [101] |
| NM_007899 | Ecm1        | 4     | Interleukin-2 receptor binding [102]     |
| NM_009401 | Tnfsf8      | 4     | Tumor necrosis factor-activated receptor activity |
| NM_176933 | Dusp4       | 4     | MAP kinase tyrosine/serine/threonine phosphatase activity |
| NM_016780 | Itgb3       | 4     | Alpha9-beta1 integrin-ADAM8 complex [103] |
| NM_010555 | Il1r2       | 4     | Interleukin-1 receptor activity [104]    |
| NM_010137 | Epas1       | 4     | DNA binding transcription factor activity [105] |
| NM_001045530 | Ccnj1 | 4     | Nucleus component                      |
| NM_019676 | Pldc1       | 4     | Phosphatidylinositol phosphate binding [106] |
| NM_001002927 | Penk | 4     | Aggressive behavior [107]               |
| NM_030887 | Jdp2        | 4     | RNA polymerase II proximal promoter sequence-specific DNA binding [108] |
| NM_025768 | Grtp1       | 4     | Rab GTPase binding                      |

Table 1. Summary of the target genes of the GATA3 and Menin with functions of the encoded proteins (based on https://www.ncbi.nlm.nih.gov/gene).
7. Conclusions

Since the human genome project was completed in 2003, the human genomic DNA database has become accessible to researchers [109]. Open access to the reference genomes of humans, mice, and other organisms encourages scientists to develop elegant technologies, including ChIP-seq and high-throughput sequencing of RNA (RNA-seq) [110]. This technique enables us to analyze the epigenetic status of each population of cells on a genome-wide scale. Many scientists have tried to use this technique to clarify the functional roles of epigenetic modifications in gene expression, particularly in the fields of developmental biology and immunology [47].

Recently, we identified several important principles between the binding positions of PcG and TrxG proteins and the gene expression [52]; a binding pattern in which PcG binds upstream and TrxG binds downstream of the transcription start site is frequently found at highly expressed genes, and a binding pattern in which PcG and TrxG bind to opposite positions is frequently found at low-expressed genes in T lymphocytes. We hope that these findings will prove useful for understanding how CD4+ T cells acquire effector functions and identifying new therapeutic targets for treating allergic diseases, such as asthma, allergic rhinitis, food allergy, and atopic dermatitis. A recently developed epigenetic editing technique using the CRISPR/Cas9 system now allows us to modify epigenetic marks in a site-specific manner [111]. In the future, we may use this technique to treat various diseases cause by epigenetic alternations.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ATAC-Seq     | assay for transposase-accessible chromatin sequencing |
| ChIP-Seq     | chromatin immunoprecipitation followed by massively parallel sequencing |
| CNS          | conserved non-coding sequence |
| H3K27me3     | trimethylated histone H3 lysine 27 |
| H3K4me3      | trimethylated histone H3 lysine 4 |
| HS           | DNase I hypersensitive site |
| IL           | interleukin |
| PcG          | Polycomb group |
| PRC          | Polycomb repressive complex |
| STAT         | signal transducer and activator of transcription |
| Th           | helper T cell |
| TrxG         | Trithorax group |
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References

[1] Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). Annual Review of Immunology. 2010;28:445-489

[2] Gu C, Wu L, Li X. IL-17 family: Cytokines, receptors and signaling. Cytokine. 2013;64(2):477-485

[3] Nakayama T, Hirahara K, Onodera A, Endo Y, Hosokawa H, Shinoda K, et al. Th2 cells in health and disease. Annual Review of Immunology. 2017;35:53-84

[4] Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000;100(6):655-669

[5] Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity. 1996;4(3):313-319

[6] Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997;89(4):587-596

[7] Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. The Journal of Biological Chemistry. 1997;272(34):21597-21603

[8] Horiuchi S, Onodera A, Hosokawa H, Watanabe Y, Tanaka T, Sugano S, et al. Genome-wide analysis reveals unique regulation of transcription of Th2-specific genes by GATA3. Journal of Immunology. 2011;186(11):6378-6389

[9] Sasaki T, Onodera A, Hosokawa H, Watanabe Y, Horiuchi S, Yamashita J, et al. Genome-wide gene expression profiling revealed a critical role for GATA3 in the maintenance of the Th2 cell identity. PLoS One. 2013;8(6):e66468. DOI: 10.1371/journal.pone.0066468

[10] Onodera A, Kiuchi M, Kokubo K, Kato M, Ogino T, Horiuchi S, et al. Menin controls the memory Th2 cell function by maintaining the epigenetic integrity of Th2 cells. Journal of Immunology. 2017;199(3):1153-1162. Copyright © [2017] The American Association of Immunologists, Inc

[11] Pai SY, Truitt ML, Ho IC. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(7):1993-1998
[12] Goenka S, Kaplan MH. Transcriptional regulation by STAT6. Immunologic Research. 2011;50(1):87-96

[13] Hebenstreit D, Wirnsberger G, Horejs-Hoeck J, Duschl A. Signaling mechanisms, interaction partners, and target genes of STAT6. Cytokine & Growth Factor Reviews. 2006;17(3):173-188

[14] Maier E, Duschl A, Horejs-Hoeck J. STAT6-dependent and -independent mechanisms in Th2 polarization. European Journal of Immunology. 2012;42(11):2827-2833

[15] Ehret GB, Reichenbach P, Schindler U, Horvath CM, Fritz S, Nabholz M, et al. DNA binding specificity of different STAT proteins. Comparison of in vitro specificity with natural target sites. The Journal of Biological Chemistry. 2001;276(9):6675-6688

[16] Nguyen VT, Benveniste EN. IL-4-activated STAT-6 inhibits IFN-gamma-induced CD40 gene expression in macrophages/microglia. Journal of Immunology. 2000;165(11):6235-6243

[17] Bennett BL, Cruz R, Lacson RG, Manning AM. Interleukin-4 suppression of tumor necrosis factor alpha-stimulated E-selectin gene transcription is mediated by STAT6 antagonism of NF-kappaB. The Journal of Biological Chemistry. 1997;272(15):10212-10219

[18] Elo LL, Jarvenpaa H, Tuomela S, Raghav S, Ahlfors H, Laurila K, et al. Genome-wide profiling of interleukin-4 and STAT6 transcription factor regulation of human Th2 cell programming. Immunity. 2010;32(6):852-862

[19] O'Shea JJ, Lahesmaa R, Vahedi G, Laurence A, Kanno Y. Genomic views of STAT function in CD4+ T helper cell differentiation. Nature Reviews. Immunology. 2011;11(4):239-250

[20] Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature. 1993;362(6417):245-248

[21] Wurster AL, Tanaka T, Grusby MJ. The biology of Stat4 and Stat6. Oncogene. 2000;19(21):2577-2584

[22] Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, et al. Essential role of Stat6 in IL-4 signalling. Nature. 1996;380(6575):627-630

[23] Cote-Sierra J, Foucras G, Guo L, Chiodetti L, Young HA, Hu-Li J, et al. Interleukin 2 plays a central role in Th2 differentiation. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(11):3880-3885

[24] Stritesky GL, Muthukrishnan R, Sehra S, Goswami R, Pham D, Travers J, et al. The transcription factor STAT3 is required for T helper 2 cell development. Immunity. 2011;34(1):39-49

[25] Mosmann TR, Coffman RL. TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology. 1989;7:145-173

[26] Reiner SL, Locksley RM. The regulation of immunity to Leishmania major. Annual Review of Immunology. 1995;13:151-177
[27] Ho IC, Tai TS, Pai SY. GATA3 and the T-cell lineage: Essential functions before and after T-helper-2-cell differentiation. Nature Reviews. Immunology. 2009;9(2):125-135

[28] Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. Annual Review of Immunology. 2009;27:485-517

[29] Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. Science. 2002;295(5553):338-342

[30] Lee HJ, Takemoto N, Kurata H, Kamogawa Y, Miyatake S, O'Garra A, et al. GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. The Journal of Experimental Medicine. 2000;192(1):105-115

[31] Pai SY, Truitt ML, Ting CN, Leiden JM, Glimcher LH, Ho IC. Critical roles for transcription factor GATA-3 in thymocyte development. Immunity. 2003;19(6):863-875

[32] Hernandez-Hoyos G, Anderson MK, Wang C, Rothenberg EV, Alberola-Ila J. GATA-3 expression is controlled by TCR signals and regulates CD4/CD8 differentiation. Immunity. 2003;19(1):83-94

[33] Yamamoto M, Ko LJ, Leonard MW, Beug H, Orkin SH, Engel JD. Activity and tissue-specific expression of the transcription factor NF-E1 multigene family. Genes & Development. 1990;4(10):1650-1662

[34] Hosoya T, Maillard I, Engel JD. From the cradle to the grave: Activities of GATA-3 throughout T-cell development and differentiation. Immunological Reviews. 2010;238(1):110-125

[35] Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, et al. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. Immunity. 1999;9(5):745-755

[36] Ouyang W, Lohning M, Gao Z, Assenmacher M, Ranganath S, Radbruch A, et al. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. Immunity. 2000;12(1):27-37

[37] Hosokawa H, Kimura MY, Shinnakasu R, Suzuki A, Miki T, Koseki H, et al. Regulation of Th2 cell development by Polycomb group gene bmi-1 through the stabilization of GATA3. Journal of Immunology. 2006;177(11):7656-7664

[38] Shinnakasu R, Yamashita M, Shinoda K, Endo Y, Hosokawa H, Hasegawa A, et al. Critical YxKxHxxRP motif in the C-terminal region of GATA3 for its DNA binding and function. Journal of Immunology. 2006;177(9):5801-5810

[39] Yamashita M, Shinnakasu R, Asou H, Kimura M, Hasegawa A, Hashimoto K, et al. Ras-ERK MAPK cascade regulates GATA3 stability and Th2 differentiation through ubiquitin-proteasome pathway. The Journal of Biological Chemistry. 2005;280(33):29409-29419

[40] Yamashita M, Ukai-Tadenuma M, Miyamoto T, Sugaya K, Hosokawa H, Hasegawa A, et al. Essential role of GATA3 for the maintenance of type 2 helper T (Th2) cytokine production and chromatin remodeling at the Th2 cytokine gene loci. The Journal of Biological Chemistry. 2004;279(26):26983-26990
Inami M, Yamashita M, Tenda Y, Hasegawa A, Kimura M, Hashimoto K, et al. CD28 costimulation controls histone hyperacetylation of the interleukin 5 gene locus in developing Th2 cells. The Journal of Biological Chemistry. 2004;279(22):23123-23133

Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nature Immunology. 2004;5(11):1157-1165

Nakayama T, Yamashita M. Critical role of the Polycomb and Trithorax complexes in the maintenance of CD4 T cell memory. Seminars in Immunology. 2009;21(2):78-83

Onodera A, Nakayama T. Epigenetics of T cells regulated by Polycomb/Trithorax molecules. Trends in Molecular Medicine. 2015;21(5):330-340

Di Croce L, Helin K. Transcriptional regulation by Polycomb group proteins. Nature Structural & Molecular Biology. 2013;20(10):1147-1155

Mohan M, Herz HM, Shilatifard A. SnapShot: Histone lysine methylase complexes. Cell. 2012;149(2):498-4e1

Steffen PA, Ringrose L. What are memories made of? How Polycomb and Trithorax proteins mediate epigenetic memory. Nature Reviews. Molecular Cell Biology. 2014;15(5):340-356

Hopkin AS, Gordon W, Klein RH, Espitia F, Daily K, Zeller M, et al. GRHL3/GET1 and trithorax group members collaborate to activate the epidermal progenitor differentiation program. PLoS Genetics. 2012;8(7):e1002829

Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, et al. Role of histone H2A ubiquitination in Polycomb silencing. Nature. 2004;431(7010):873-878

Tumes DJ, Onodera A, Suzuki A, Shinoda K, Endo Y, Iwamura C, et al. The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. Immunity. 2013;39(5):819-832

Onodera A, Yamashita M, Endo Y, Kuwahara M, Tofukuji S, Hosokawa H, et al. STAT6-mediated displacement of polycomb by trithorax complex establishes long-term maintenance of GATA3 expression in T helper type 2 cells. The Journal of Experimental Medicine. 2010;207(11):2493-2506

Onodera A, Tumes DJ, Watanabe Y, Hirahara K, Kaneda A, Sugiyama F, et al. Spatial interplay between polycomb and trithorax complexes controls transcriptional activity in T lymphocytes. Molecular and Cellular Biology. 2015;35(22):3841-3853

Schuettengruber B, Martinez AM, Iovino N, Cavalli G. Trithorax group proteins: Switching genes on and keeping them active. Nature Reviews. Molecular Cell Biology. 2011;12(12):799-814

Matkar S, Thiel A, Hua X. Menin: A scaffold protein that controls gene expression and cell signaling. Trends in Biochemical Sciences. 2013;38(8):394-402

Balogh K, Raczk, Patocs A, Hunyady L. Menin and its interacting proteins: Elucidation of menin function. Trends in Endocrinology and Metabolism. 2006;17(9):357-364
Nakata Y, Brignier AC, Jin S, Shen Y, Rudnick SI, Sugita M, et al. C-Myb, Menin, GATA-3, and MLL form a dynamic transcription complex that plays a pivotal role in human T helper type 2 cell development. Blood. 2010;116(8):1280-1290

Zhang JA, Mortazavi A, Williams BA, Wold BJ, Rothenberg EV. Dynamic transformations of genome-wide epigenetic marking and transcriptional control establish T cell identity. Cell. 2012;149(2):467-482

Margueron R, Reinberg D. The polycomb complex PRC2 and its mark in life. Nature. 2011;469(7330):343-349

Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and Il4 locus accessibility. Annual Review of Immunology. 2006;24:607-656

Scheinman EJ, Avni O. Transcriptional regulation of GATA3 in T helper cells by the integrated activities of transcription factors downstream of the interleukin-4 receptor and T cell receptor. The Journal of Biological Chemistry. 2009;284(5):3037-3048

Deaton AM, Webb S, Kerr AR, Illingworth RS, Guy J, Andrews R, et al. Cell type-specific DNA methylation at intragenic CpG islands in the immune system. Genome Research. 2011;21(7):1074-1086

Wei L, Vahedi G, Sun HW, Watford WT, Takatori H, Ramos HL, et al. Discrete roles of STAT4 and STAT6 transcription factors in tuning epigenetic modifications and transcription during T helper cell differentiation. Immunity. 2010;32(6):840-851

Shih HY, Sciume G, Mikami Y, Guo L, Sun HW, Brooks SR, et al. Developmental acquisition of regulomes underlies innate lymphoid cell functionality. Cell. 2016;165(5):1120-1133

Yamashita M, Hirahara K, Shinnakasu R, Hosokawa H, Norikane S, Kimura MY, et al. Crucial role of MLL for the maintenance of memory T helper type 2 cell responses. Immunity. 2006;24(5):611-622

Liu J, Cao L, Chen J, Song S, Lee IH, Quijano C, et al. Bmi1 regulates mitochondrial function and the DNA damage response pathway. Nature. 2009;459(7245):387-392

Koyanagi M, Baguet A, Martens J, Margueron R, Jenuwein T, Bix M. EZH2 and histone 3 trimethyl lysine 27 associated with Il4 and Il13 gene silencing in Th1 cells. The Journal of Biological Chemistry. 2005;280(36):31470-31477

Allan RS, Zueva E, Cammas F, Schreiber HA, Masson V, Belz GT, et al. An epigenetic silencing pathway controlling Thelper 2 cell lineage commitment. Nature. 2012;487(7406):249-253

Yagi R, Zhu J, Paul WE. An updated view on transcription factor GATA3-mediated regulation of Th1 and Th2 differentiation. International Immunology. 2011;23(7):415-420

Yu M, Riva L, Xie H, Schindler Y, Moran TB, Cheng Y, et al. Insights into GATA-1-mediated gene activation versus repression via genome-wide chromatin occupancy analysis. Molecular Cell. 2009;36(4):682-695

Takemoto N, Kamogawa Y, Jun Lee H, Kurata H, Arai KI, O’Garra A, et al. Cutting edge: Chromatin remodeling at the IL-4/IL-13 intergenic regulatory region for Th2-specific cytokine gene cluster. Journal of Immunology. 2000;165(12):6687-6691
[71] Takemoto N, Arai K, Miyatake S. Cutting edge: The differential involvement of the N-finger of GATA-3 in chromatin remodeling and transactivation during Th2 development. Journal of Immunology. 2002;169(8):4103-4107

[72] Agarwal S, Avni O, Rao A. Cell-type-restricted binding of the transcription factor NFAT to a distal IL-4 enhancer in vivo. Immunity. 2000;12(6):643-652

[73] Yamashita M, Ukai-Tadenuma M, Kimura M, Omori M, Inami M, Taniguchi M, et al. Identification of a conserved GATA3 response element upstream proximal from the interleukin-13 gene locus. The Journal of Biological Chemistry. 2002;277(44):42399-42408

[74] Tanaka S, Motomura Y, Suzuki Y, Yagi R, Inoue H, Miyatake S, et al. The enhancer HS2 critically regulates GATA-3-mediated Il4 transcription in T(H)2 cells. Nature Immunology. 2011;12(1):77-85

[75] Takemoto N, Koyano-Nakagawa N, Yokota T, Arai N, Miyatake S, Arai K. Th2-specific DNase I-hypersensitive sites in the murine IL-13 and IL-4 intergenic region. International Immunology. 1998;10(12):1981-1985

[76] Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: Regulation by cis elements and epigenetics. Immunity. 2006;24(4):369-379

[77] Mohrs M, Blankespoor CM, Wang ZE, Loots GG, Afzal V, Hadeiba H, et al. Deletion of a coordinate regulator of type 2 cytokine expression in mice. Nature Immunology. 2001;2(9):842-847

[78] Monticelli S, Lee DU, Nardone J, Bolton DL, Rao A. Chromatin-based regulation of cytokine transcription in Th2 cells and mast cells. International Immunology. 2005;17(11):1513-1524

[79] Wei G, Abraham BJ, Yagi R, Jothi R, Cui K, Sharma S, et al. Genome-wide analyses of transcription factor GATA3-mediated gene regulation in distinct T cell types. Immunity. 2011;35(2):299-311

[80] Lee DU, Agarwal S, Rao A. Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. Immunity. 2002;16(5):649-660

[81] Solymar DC, Agarwal S, Bassing CH, Alt FW, Rao A. A 3′ enhancer in the IL-4 gene regulates cytokine production by Th2 cells and mast cells. Immunity. 2002;17(1):41-50

[82] Vijayanand P, Seumois G, Simpson LJ, Abdul-Wajid S, Baumjohann D, Panduro M, et al. Interleukin-4 production by follicular helper T cells requires the conserved Il4 enhancer hypersensitivity site V. Immunity. 2012;36(2):175-187

[83] Zhu J, Cote-Sierra J, Guo L, Paul WE. Stat5 activation plays a critical role in Th2 differentiation. Immunity. 2003;19(5):739-748

[84] Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity. 2009;30(1):155-167

[85] Lee HJ, O’Garra A, Arai K, Arai N. Characterization of cis-regulatory elements and nuclear factors conferring Th2-specific expression of the IL-5 gene: A role for a GATA-binding protein. Journal of Immunology. 1998;160(5):2343-2352
[86] Schwenger GT, Fournier R, Kok CC, Mordvinov VA, Yeoman D, Sanderson CJ. GATA-3 has dual regulatory functions in human interleukin-5 transcription. The Journal of Biological Chemistry. 2001;276(51):48502-48509

[87] Kishikawa H, Sun J, Choi A, Miaw SC, Ho IC. The cell type-specific expression of the murine IL-13 gene is regulated by GATA-3. Journal of Immunology. 2001;167(8):4414-4420

[88] Iseki M, Takaki S, Takatsu K. Molecular cloning of the mouse APS as a member of the Lnk family adaptor proteins. Biochemical and Biophysical Research Communications. 2000;272(1):45-54

[89] Bello NF, Lamsoul I, Heuze ML, Metais A, Moreaux G, Calderwood DA, et al. The E3 ubiquitin ligase specificity subunit ASB2beta is a novel regulator of muscle differentiation that targets filamin B to proteasomal degradation. Cell Death and Differentiation. 2009;16(6):921-932

[90] Zhang M, Park SM, Wang Y, Shah R, Liu N, Murmann AE, et al. Serine protease inhibitor 6 protects cytotoxic T cells from self-inflicted injury by ensuring the integrity of cytotoxic granules. Immunity. 2006;24(4):451-461

[91] Schaefer G, Venkataraman C, Schindler U. Cutting edge: FISP (IL-4-induced secreted protein), a novel cytokine-like molecule secreted by Th2 cells. Journal of Immunology. 2001;166(10):5859-5863

[92] Zingoni A, Soto H, Hedrick JA, Stoppacciaro A, Storlazzi CT, Sinigaglia F, et al. The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. Journal of Immunology. 1998;161(2):547-551

[93] Casey LS, Lichtman AH, Boothby M. IL-4 induces IL-2 receptor p75 beta-chain gene expression and IL-2-dependent proliferation in mouse T lymphocytes. Journal of Immunology. 1992;148(11):3418-3426

[94] Han S, Nam J, Li Y, Kim S, Cho SH, Cho YS, et al. Regulation of dendritic spines, spatial memory, and embryonic development by the TANC family of PSD-95-interacting proteins. The Journal of Neuroscience. 2010;30(45):15102-15112

[95] Motomura Y, Kitamura H, Hijikata A, Matsunaga Y, Matsumoto K, Inoue H, et al. The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in CD4+ T cells. Nature Immunology. 2011;12(5):450-459

[96] Zmrzljak UP, Korencic A, Kosir R, Golicnik M, Sassone-Corsi P, Rozman D. Inducible cAMP early repressor regulates the period 1 gene of the hepatic and adrenal clocks. The Journal of Biological Chemistry. 2013;288(15):10318-10327

[97] McCoy KL, Traynelis SF, Hepler JR. PAR1 and PAR2 couple to overlapping and distinct sets of G proteins and linked signaling pathways to differentially regulate cell physiology. Molecular Pharmacology. 2010;77(6):1005-1015

[98] O'Shaughnessy PJ, Mannan MA. Development of cytochrome P-450 side chain cleavage mRNA levels in neonatal ovaries of normal and hypogonadal (hpg) mice. Molecular and Cellular Endocrinology. 1994;104(2):133-138
Borchers AG, Hufton AL, Eldridge AG, Jackson PK, Harland RM, Baker JC. The E3 ubiquitin ligase GREUL1 anteriorizes ectoderm during Xenopus development. Developmental Biology. 2002;251(2):395-408

Tarabykina S, Kriaевska M, Scott DJ, Hill TJ, Lafitte D, Derrick PJ, et al. Heterocomplex formation between metastasis-related protein S100A4 (Mts1) and S100A1 as revealed by the yeast two-hybrid system. FEBS Letters. 2000;475(3):187-191

Gross I, Bassit B, Benezra M, Licht JD. Mammalian sprouty proteins inhibit cell growth and differentiation by preventing ras activation. The Journal of Biological Chemistry. 2001;276(49):46460-46468

Li Z, Zhang Y, Liu Z, Wu X, Zheng Y, Tao Z, et al. ECM1 controls T(H)2 cell egress from lymph nodes through re-expression of S1P(1). Nature Immunology. 2011;12(2):178-185

Rao H, Lu G, Kajiya H, Garcia-Palacios V, Kurihara N, Anderson J, et al. Alpha(9)beta1: A novel osteoclast integrin that regulates osteoclast formation and function. Journal of Bone and Mineral Research. 2006;21(10):1657-1665

Zheng Y, Humphry M, Maguire JJ, Bennett MR, Clarke MC. Intracellular interleukin-1 receptor 2 binding prevents cleavage and activity of interleukin-1alpha, controlling necrosis-induced sterile inflammation. Immunity. 2013;38(2):285-295

Elver G, Kappel A, Heidenreich R, Englmeier U, Lanz S, Acker T, et al. Cooperative interaction of hypoxia-inducible factor-2alpha (HIF-2alpha) and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (Flk-1). The Journal of Biological Chemistry. 2003;278(9):7520-7530

Kim NY, Ahn SJ, Kim MS, Seo JS, Kim BS, Bak HJ, et al. PLC-delta1-lf, a novel N-terminal extended phospholipase C-delta1. Gene. 2013;528(2):170-177

Konig M, Zimmer AM, Steiner H, Holmes PV, Crawley JN, Brownstein MJ, et al. Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature. 1996;383(6600):535-538

Jin C, Ugai H, Song J, Murata T, Nili F, Sun K, et al. Identification of mouse Jun dimerization protein 2 as a novel repressor of ATF-2. FEBS Letters. 2001;489(1):34-41

Collins FS, Green ED, Guttmacher AE, Guyer MS. A vision for the future of genomics research. Nature. 2003;422(6934):835-847

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, et al. High-resolution profiling of histone methylations in the human genome. Cell. 2007;129(4):823-837

Thakore PI, D’Ippolito AM, Song L, Safi A, Shivakumar NK, Kabadi AM, et al. Highly specific epigenome editing by CRISPR-Cas9 repressors for silencing of distal regulatory elements. Nature Methods. 2015;12(12):1143-1149
