Chapter 10

The Interplay between Transcription Factors and Epigenetic Modifications in Th2 Cells

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Additional information is available at the end of the chapter

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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 TTCNNNNGAA, 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 TxrG 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)

[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. 1998;**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 YxKxHxxxRP 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

[41] 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

[42] 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

[43] 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

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

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

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

[47] 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

[48] 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

[49] 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

[50] 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

[51] 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

[52] 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

[53] 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

[54] 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

[55] Balogh K, Racz K, Patocs A, Hunyady L. Menin and its interacting proteins: Elucidation of menin function. Trends in Endocrinology and Metabolism. 2006;**17**(9):357-364

[56] 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

[57] 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

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

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

[60] 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

[61] 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

[62] 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

[63] 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

[64] 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

[65] 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

[66] 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

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

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

[69] 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

[70] 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

[99] 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

[100] Tarabykina S, Kriajevska 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

[101] 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

[102] 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

[103] Rao H, Lu G, Kajiya H, Garcia-Palacios V, Kurihara N, Anderson J, et al. Alpha9beta1: A novel osteoclast integrin that regulates osteoclast formation and function. Journal of Bone and Mineral Research. 2006;**21**(10):1657-1665

[104] 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

[105] Elvert 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

[106] 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

[107] 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

[108] 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

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

[110] 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

[111] 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

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