



Chromatin Accessibility and Interactions in the Transcriptional Regulation of T Cells

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During T cell differentiation and activation, specific stimuli, and a network of transcription factors (TFs) are involved in orchestrating chromatin accessibility, establishing enhancer-promoter interactions, and regulating gene expression. Over the past few years, there have been new insights into how chromatin interactions coordinate differentiation during T cell development and how regulatory elements are programmed to allow T cells to differentially respond to distinct stimuli. In this review, we discuss recent advances related to the roles of TFs in establishing the regulatory chromatin landscapes that orchestrate T cell development and differentiation. In particular, we focus on the role of TFs (e.g., TCF-1, BCL11B, PU.1, STAT3, STAT5, AP-1, and IRF4) in mediating chromatin accessibility and interactions and in regulating gene expression in T cells, including gene expression that is dependent on IL-2 and IL-21. Furthermore, we discuss the state of knowledge on enhancer-promoter interactions and how autoimmune disease risk variants can be linked to molecular functions of putative target genes.

Keywords: transcription factors, chromatin accessibility, T cells, STAT5, ChIA-PET, chromatin interactions

INTRODUCTION

Transcriptomic profiles determine the phenotype and function of cells, and this process is tightly controlled by various transcription factors (TFs), epigenetics, and chromatin interactions to define transcriptional patterns in response to cellular signals. More specifically, control of gene expression depends not only on the binding of sequence-specific TFs to target DNA sequences, but also on chromatin accessibility, which is controlled by the proper packaging of DNA/nucleosomes (chromatin) within the nucleus, leading to the arrangement of the genome into distinct spatial structures. Differences in chromatin composition can determine gene expression profiles in cells by providing relative accessibility (open or closed) of key regions to TFs that bind to DNA. Within the immune system, upon cellular stimulation by extracellular signals (e.g., via the T cell receptor (TCR) or cytokines), chromatin composition is modified through the concerted actions of signal-specific TFs and chromatin modifiers via a dynamic process. Naturally occurring genetic mutations in binding sites for TFs that alter the chromatin landscape can potentially disrupt or establish chromatin interactions, thereby resulting in altered gene expression profiles, and predisposing to cancer, autoimmune disease, allergy, immunodeficiency, or other immune disorders. In this review, we focus on the cooperative actions of TFs that play critical roles in shaping the chromatin landscape and accessibility in early and mature T cell development, and how these dynamic changes can alter gene expression profiles.

TRANSCRIPTION FACTORS THAT ESTABLISH CHROMATIN LANDSCAPE DURING EARLY T CELL DEVELOPMENT

Lineage specific transcription factors (LSTFs) or master regulators are expressed at critical times during lymphoid development or differentiation, and they contribute to cell type determination. During the development of T cells in the thymus, master regulators such as TCF-1, BCL11B, GATA3, PU.1, and RUNX family TFs are critical for T-lineage commitment (**Figure 1**) (1–3). To properly regulate gene expression, TFs must recognize and bind to their sequence-specific DNA binding sites (motifs). Access to regulatory regions in the genome is tightly controlled by chromatin structure. Genome-wide analysis using Hi-C technology, a method coupling 3C (Chromosome Conformation Capture) methodology with high-throughput DNA sequencing (4), has revealed that the genome can be divided into spatially separated regions or “compartments,” which are composed of smaller Topologically Associating Domains (TADs) that can be brought into close proximity to each other by chromatin looping (4–6).

During early T cell development, hematopoietic stem cells develop into T cell progenitor cells, termed CD4⁺CD8⁻ or double-negative (DN) thymocytes, which can then progress through four stages of maturation (denoted DN1, DN2a/b, DN3a/b, and DN4 cells). T cell commitment occurs at the DN2a to DN2b developmental transitional stages (1–3), and there is a key checkpoint termed β -selection at the CD25⁺CD44⁻ DN3a to DN3b/4 maturation step, with gene rearrangement of the TCR β chain. Following β -selection, T cells further mature into CD4⁺CD8⁺ double-positive (DP) cells, which express both CD4 and CD8 (3). Some evidence suggests that dynamic changes in chromatin modifications and transcription are associated with T cell development (7), but it is not clear if there are genome-wide modifications in higher-order chromatin structures and whether such structures are required to establish T cell identity. GATA-3 is essential throughout the early T cell developmental stages, including for T cell commitment, β -selection, and CD4⁺ cell fate choice during positive selection (8).

The IL-7/IL-7R axis plays major roles in the survival of DN thymocytes during early T-cell development (9, 10). IL-7-signaling activates major signaling pathways, including JAK1/JAK3-STAT5 and PI 3-kinase, and Y449 of the IL-7R is part of a YxxM motif and can mediate not only recruitment of STAT5 but also the p85 subunit of PI 3-kinase (11). IL-7-mediated signaling results in the induction of anti-apoptotic BCL-2 and MCL-1 proteins but the decreased expression of pro-apoptotic proteins (9), and STAT5 has been implicated in the regulation of expression of BCL-2 (12). IL-7-mediated STAT5 activation controls chromatin accessibility and rearrangement of the TCR γ locus (13, 14). In addition to its activation of STAT5, IL-7 was reported to activate NFATc1, with this serving as an alternative signaling pathway that cooperates with STAT5 to guide thymocyte development (15). Thus, IL-7-mediated transcriptional activation serves important roles in T cell development.

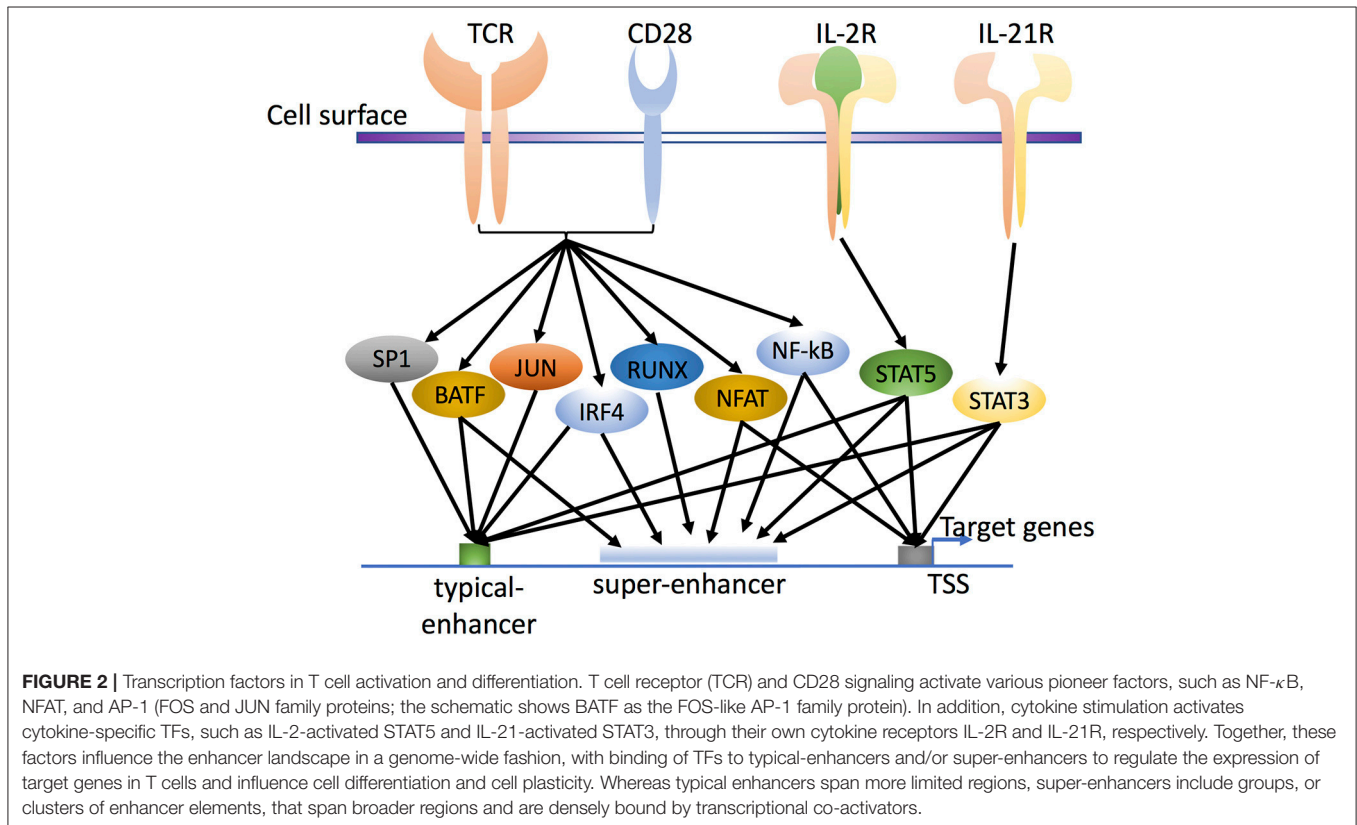
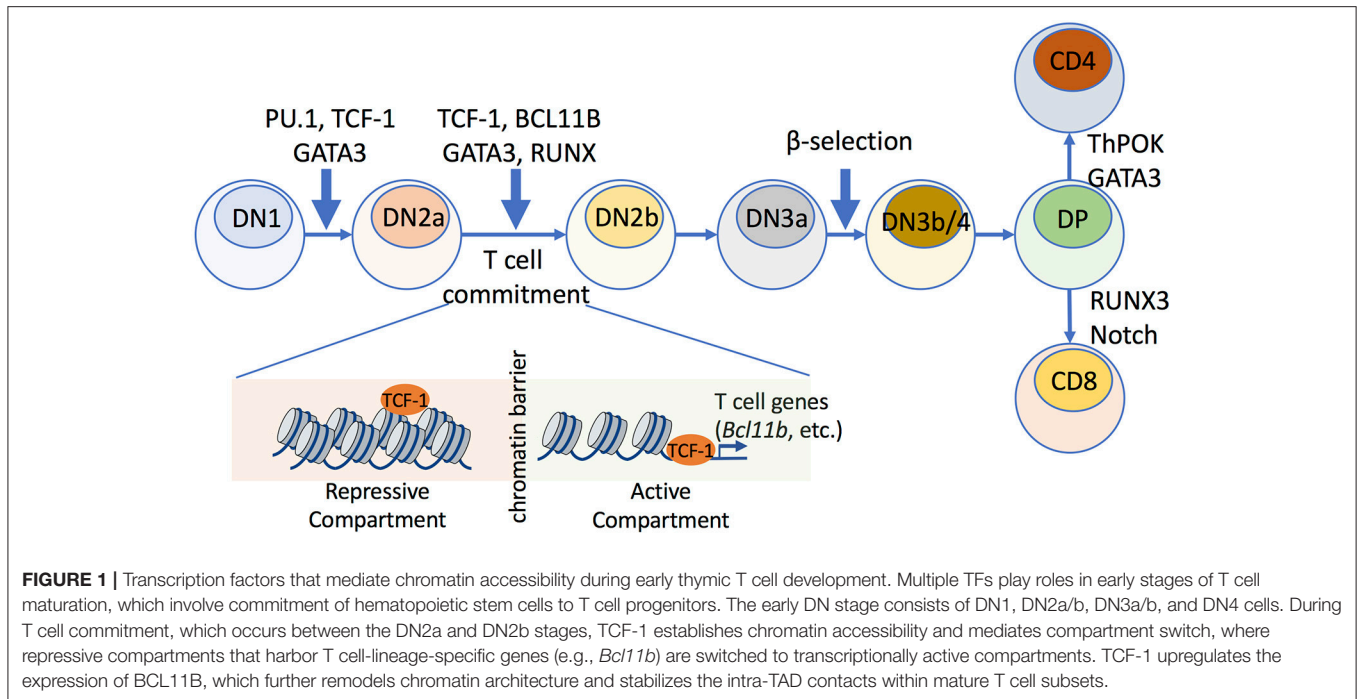
Recent studies have provided further insights into the mechanisms by which two TFs, TCF-1, and BCL11B, drive T cell differentiation by modifying the nuclear architecture to generate distinct chromatin landscapes (16, 17). Chromatin accessibility across distinct stages of T cell development was profiled using single-cell DNase-Seq (DNase I hypersensitive sites sequencing) (18, 19) and ATAC-Seq (Assay for Transposase Accessible Chromatin combined with DNA sequencing) (20, 21) to reveal that dynamic modifications in chromatin accessibility appeared genome-wide during T cell differentiation (16, 17). Strikingly, different stages of chromatin accessibility were observed as developing cells progress during T cell commitment (**Figure 1**). BCL11B, a critical regulator of T cell commitment, was found to play critical roles in maintaining higher-order chromatin structures and was associated with increased chromatin interactions during T cell lineage commitment (17). Furthermore, at early stages of T cell differentiation, TCF-1 was significantly enriched at accessible chromatin that was associated with T cell-lineage-specific gene loci (16). Mice deficient in *Tcf7*, which encodes TCF-1, cannot properly establish the open chromatin landscape of normal T cells, suggesting that the initiation of chromatin remodeling was TCF-1-dependent, and this was particularly evident at the *Bcl11b* locus (**Figure 1**).

PU.1 (encoded by *Spi1/Sfpi1*) was shown to function as a specialized nucleosome-binding transcription factor during the DN1-DN2 transition (22, 23), and this factor can bind to closed chromatin and rapidly open genomic sites. Specifically, ChIP-Seq (Chromatin Immunoprecipitation combined with high-throughput DNA sequencing) (24, 25) and ATAC-Seq analysis showed that the chromatin of selected regions is opened by PU.1 within 24 h (22, 23), suggesting that PU.1 acts as a “pioneer factor” to remodel chromatin structure during early T-cell development.

TRANSCRIPTION FACTORS THAT MEDIATE CHROMATIN ACCESSIBILITY DURING T CELL DIFFERENTIATION

During CD4⁺ T cell differentiation, a range of pioneer factors are activated to shape the epigenetic landscape and regulate chromatin accessibility for TFs (26–29). T cell activation requires antigen signaling via the TCR and co-stimulation with CD28, resulting in nuclear translocation of a number of TFs, including AP-1 and NFAT (**Figure 2**). Interestingly, BATE, a FOS-like AP-1 family transcription factor, and IRF4 were shown to function as pioneer factors that could regulate chromatin accessibility during differentiation of Th17 (30) and CD8⁺ T cells (31).

Following TCR stimulation, cytokines including IL-2 and IL-21 are also produced and in turn activate and induce the nuclear translocation of STAT proteins (e.g., STAT5 and STAT3) through cytokine receptors IL-2R and IL-21R (**Figure 2**), and these factors collectively help to prepare the T cell chromatin landscape. Interestingly, both STAT dimers and tetramers can form, with STAT5 tetramers being critical for the normal development and expansion of key immune populations (32, 33). Cytokines secreted by immune cells can also drive T helper cell



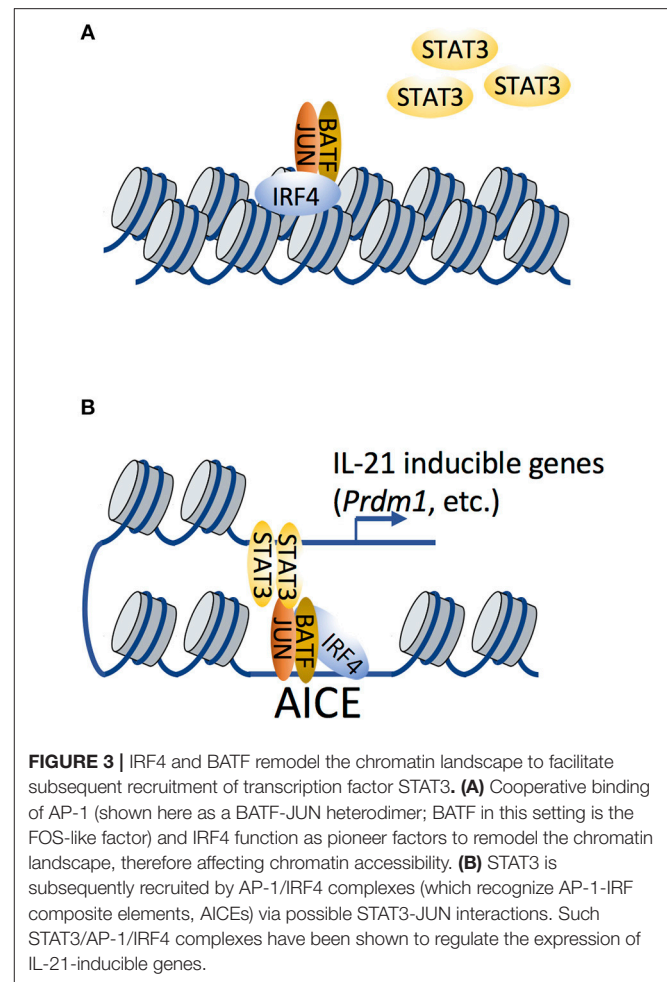
differentiation. For example, IL-12, IL-4, and IL-6 drive Th1, Th2, and Th17 differentiation, respectively, with critical roles for IL-2 in promoting (Th1, Th2, Th9, Treg) or inhibiting (Th17 or

T follicular helper [T_{fh}] cell) differentiation and often opposing actions for IL-21 (34–37). All of these cytokines are 4- α helical bundle type 1 cytokines that use the JAK-STAT pathway

as a major signaling pathway to transduce extracellular cytokine signals into the cell and regulate expression of corresponding genes (37–39). Interestingly, of these cytokines, IL-2, IL-4, and IL-21 signal via receptors that belong to the common cytokine receptor γ chain (γ_c , also known as the IL-2 receptor γ chain IL-2R γ , or CD132) family of cytokines (40, 41). During CD4⁺ T cell differentiation, STATs can have major impact on the activation of lineage-specific enhancers and the suppression of enhancers associated with alternative cell fates. For example, STATs can shape the active enhancer landscape in Th1 and Th2 cells (42, 43) in the presence of different cytokine signals, with IL-12/STAT4 and IFN- γ /STAT1 driving Th1 and IL-4/STAT6 driving Th2 differentiation, respectively. In addition, however, IL-2 via STAT5 serves a key role and primes T cells for responsiveness to IL-12 and IL-4 and Th differentiation. For Th1 differentiation, IL-2 via STAT5 augments expression of IL-12R β 2 and TBET (44) and for Th2 differentiation, it augments expression of IL-4R α (45) and IL-4 (46–48), with IL-2-induced STAT5 kinetically binding earlier to the *Il4ra* than to the *Il4* locus. Interestingly, IL-2-activated STAT5 binding was shown to augment chromatin accessibility at the *Il4* locus (46). IL-2 via STAT5 also inhibits Th17 differentiation (49, 50), potentially by several mechanisms, including a direct IL-2-STAT5 competition with IL-6-STAT3 (49, 51), the inhibition by IL-2-STAT5 of gp130 expression and by IL-2-mediated induction of TBET, which interacts with RUNX1, potentially limiting the required RUNX1-ROR γ t interaction (44). Moreover, IL-2-STAT5 drives Th9 differentiation (52) and limits Tfh differentiation (53, 54) whereas, IL-21-STAT3 has an opposing effect (52, 55, 56). As compared to pioneer factors, cytokines that influence Th differentiation have less profound effects on the epigenetic landscape.

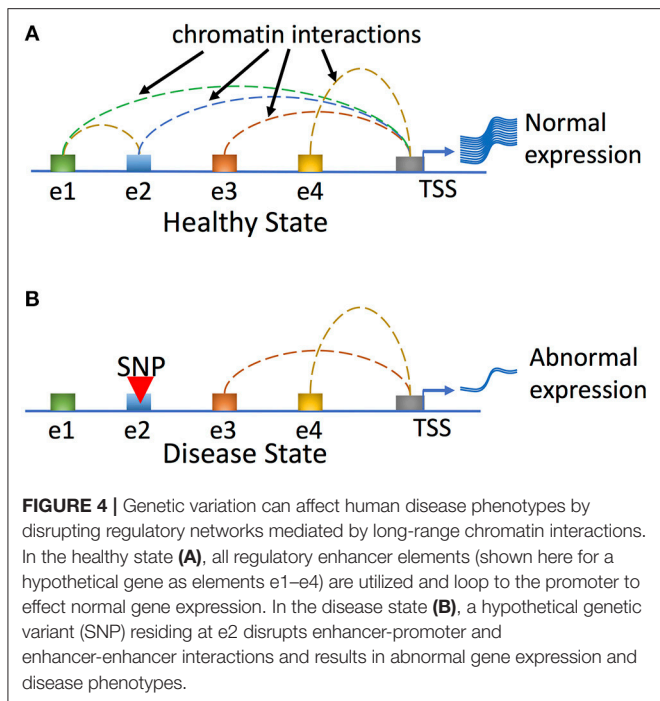
INTERPLAY OF CHROMATIN AND TRANSCRIPTION FACTORS AP-1, IRF4, AND STAT3 IN T CELLS

BATF and IRF4 were shown to functionally cooperate and recognize specific AP-1-IRF composite elements (AICEs) mainly in T cells and dendritic cells (57–59) and these factors cooperate to regulate chromatin accessibility during the differentiation of Th17 (30) and CD8⁺ T (31) cells (Figure 3). FAIRE-Seq (Chromatin accessibility analysis using formaldehyde-assisted isolation of regulatory elements sequencing) (60) revealed that the loss of BATF or IRF4 in Th0 or Th17 cells had little if any effect on genomic loci already accessible in naive cells, but most loci with inducible accessibility exhibited marked reductions in *Batf*- or *Irf4*-deficient mice compared to wild-type cells, suggesting that IRF4 and BATF remodel the chromatin landscape and potentially facilitate subsequent recruitment of TFs involved in regulating expression of Th17-relevant genes (30). Enhancer occupancy by AP-1/IRF4 complexes correlates with sensitivity of gene expression in response to TCR signaling (61, 62), so that genes with low-affinity or high-affinity AICE-dependent enhancers are induced at lower or higher TCR signal strength, respectively. IRF4 alone was also shown to be induced in a manner dependent on TCR affinity, and as a dose-dependent



regulator of the metabolic function of activated T cells (63). BATF is also a key regulator of early effector CD8⁺ T cell differentiation (31), and BATF-deficient CD8⁺ T cells are profoundly defective in their ability to undergo naive to effector differentiation and proliferative expansion. Moreover, BATF and IRF1 are induced early during *in vitro* regulatory T (Treg) cell differentiation and act as pioneer factors for the differentiation of type 1 Treg (Tr1) cells (64). BACH2, like AP-1 factors that contain a bZIP domain, can regulate CD8⁺ T cell differentiation by controlling the access of AP-1 factors to enhancers, thus limiting the expression of TCR-driven genes by attenuating the availability of AP-1 sites to JUN family TFs (65, 66).

IRF4 often cooperates with STAT3 in modulating IL-21-dependent gene expression in Tfh and Th17 cells (30, 58, 67). Given that STAT3 can physically bind to c-JUN (68), it is reasonable to hypothesize that STAT3 can be recruited by BATF-JUN-IRF4 complexes via STAT3-JUN interactions (Figure 3). ChIP-Seq analysis revealed that IL-21-induced STAT3 binding was dramatically diminished in *Irf4*^{-/-} CD4⁺ T cells compared to WT cells (67), suggesting that it was IRF4-dependent. It is possible that STAT3 directly binds to IRF4, or that its binding is dependent on chromatin accessibility that is pre-patterned



by IRF4 to facilitate the subsequent recruitment of STAT3 to AP-1. However, STAT3 binding motifs are not enriched in genomic proximity to AICEs (58, 69), suggesting that STAT3-IRF4 association may also occur via long-range chromatin interactions, a hypothesis that remains to be experimentally validated.

T CELL RESPONSES TO DIFFERENT STIMULI INCLUDING IL-2 AND IL-21 AND THE ROLE OF SUPER-ENHANCERS

After antigen encounter, CD4⁺ T cells are activated and secrete cytokines including IL-2 and IL-21, which regulate immune cell differentiation and effector functions by differentially activating specific STAT proteins that recognize and bind to γ -interferon-activated sequence (GAS) motifs. IL-2 potently activates STAT5, whereas IL-21 primarily activates STAT3. This differential STAT activation leads to differential gene expression by these cytokines. It is established that STAT proteins are critical components of cytokine-activated enhancers, but recently their roles related to super-enhancers (70) and their abilities to fine-tune gene expression (71) have been elucidated, with, for example, greater IL-2-inducibility of genes with STAT5-based super-enhancers, as compared to STAT5-based typical enhancers (71). As opposed to typical enhancers, where factor binding occurs in more limited regions, super-enhancers (also known as stretched or clustered enhancers) (70, 72, 73) represent groups of putative enhancers in close genomic proximity that span broader regions (Figure 2), are densely bound by transcriptional coactivators, and usually are associated with high levels of the active chromatin mark

histone H3 lysine 27 acetylation (H3K27Ac). Although super-enhancers were originally recognized in the setting of master regulator genes (74) and genes associated with cell identity, STAT5- and STAT3-dependent super-enhancers have now also been shown to exist and to regulate gene expression in a cytokine- and context-specific manner (71). Chromatin interaction analysis using paired-end tag sequencing (ChIA-PET) (75, 76) revealed that IL-2-activated STAT5 can influence RNA Polymerase II (RNA Pol II)-based chromatin interactions, with looping anchor sites in proximity to STAT5 binding sites. Moreover, CRISPR-Cas9 (77, 78) genome editing was used to generate mutant mice in order to functionally analyze the STAT5-bound super-enhancer containing gene, *Il2ra*, *in vivo*. When three of the super-enhancer elements were separately deleted, each exhibited defective expression of IL-2R α , indicating that each enhancer element contributed to IL-2-induced IL-2R α expression and that these elements were not functionally redundant (71). These observations provide insights into the mechanism underlying the regulation of IL-2 target genes. Interestingly, IL-2-based super-enhancers included not only positive regulators of signaling, such as *Il2ra*, but also negative regulators such as SOCS family proteins (e.g., *Cish*), revealing that super-enhancers are critical for both the positive and negative regulation of IL-2 signaling (71).

Similar to the mouse *Il2ra* gene, human *IL2RA* also has a similar super-enhancer that is densely bound by STAT5, and some of the enhancer elements are highly conserved in both mouse and human, consistent with an evolutionarily conserved mode of gene regulation (71, 79, 80). Interestingly, tiled CRISPR activation (CRISPRa) (81) was used to identify several CRISPRa-responsive elements with chromatin features of stimulus-responsive enhancers, including an *IL2RA* enhancer that contains a non-coding autoimmunity risk variant (80) that is conserved between humans and mice. Mutating this element in mice did not completely block *Il2ra* gene expression but rather delayed gene activation in response to TCR stimulation, indicating that the kinetics of *Il2ra* gene expression are important. This mutation skewed polarization of naive T cells from Treg cells toward pro-inflammatory Th17 cells, which elucidates its role in autoimmune disease (80).

ENHANCER-PROMOTER INTERACTIONS AND AUTOIMMUNE DISEASE-ASSOCIATED SNPs

Gene expression is regulated via complex interactions between promoters and long-range regulatory elements, and disruption of chromatin interactions by mutations (e.g., SNPs or INDELs) may result in altered target gene expression that leads to disease development (Figure 4). Another study correlated histone modification of H3K27ac with active enhancers and promoters and furthermore analyzed protein-centric chromatin interactions by utilizing HiChIP, chromatin immunoprecipitation (ChIP) and Hi-C assays (82). By generating enhancer-promoter contacts in primary naive CD4⁺ T cells, Treg cells, and Th17 cells, chromatin loops were identified that were shared by all three cell types (82).

Strikingly, the majority of these chromatin interaction anchors were associated with enhancers or promoters. Furthermore, autoimmune disease-associated variants in intergenic regions could interact with multiple target genes, providing insights into the functional interrogation of disease associated genetic variants; however, further high-resolution chromatin interactions in various cell types are needed to better explain how connections between variants and genes can be translated into molecular and cellular functions.

CONCLUDING COMMENTS

In summary, studies of the transcriptional and epigenetic regulation of T cells have identified several mechanisms of cross-regulation between TFs, chromatin modifiers, and the pre-existing chromatin landscape. The interactions between chromatin and TFs are influenced by a range of stimuli, including TCR and cytokine signals. Transcription factors are important for cell function, and they collaborate combinatorically with other factors to influence gene regulation. Their binding to DNA depends on epigenetic landscapes, and their function may

depend on chromatin interactions to juxtapose distal regulatory elements with gene promoters. The ability of cytokine-activated proteins to modify nucleosome packing and influence histone modifications allows them to control developmental processes. The gene regulatory networks that determine T cell development are broad and involve chromatin accessibility, epigenetic status, and distant chromatin interactions in both time- and context-dependent manners. Our evolving understanding of gene regulatory networks will help to comprehensively link genetic variants to putative gene targets, furthering our understanding of molecular mechanisms for a range of immune diseases. Achieving a deeper understanding of the mechanisms involved has now been greatly facilitated by genetic manipulations including CRISPR/Cas9 gene editing but still awaits other advances, such as the ability to comprehensively study single cells in real time.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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