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# Chromatin-Modifying Enzymes in T Cell Development

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## Keywords

chromatin, histone, T cell development, methylation, acetylation, ubiquitination

## Abstract

T cell development involves stepwise progression through defined stages that give rise to multiple T cell subtypes, and this is accompanied by the establishment of stage-specific gene expression. Changes in chromatin accessibility and chromatin modifications accompany changes in gene expression during T cell development. Chromatin-modifying enzymes that add or reverse covalent modifications to DNA and histones have a critical role in the dynamic regulation of gene expression throughout T cell development. As each chromatin-modifying enzyme has multiple family members that are typically all coexpressed during T cell development, their function is sometimes revealed only when two related enzymes are concurrently deleted. This work has also revealed that the biological effects of these enzymes often involve regulation of a limited set of targets. The growing diversity in the types and sites of modification, as well as the potential for a single enzyme to catalyze multiple modifications, is also highlighted.

## INTRODUCTION

The process of differentiation results from the induction of lineage-specific genes along with silencing of genes for alternative lineages. The establishment of specific patterns of gene expression is driven by changes in the structure of chromatin that determine the extent of transcription at specific genomic locations. This process has been particularly well studied in T cell development in which changes in chromatin accompany changes in gene expression as cells progress from an uncommitted progenitor through successive stages/checkpoints that ultimately give rise to multiple T cell subpopulations. The importance of chromatin-modifying enzymes during thymocyte development is the focus of this review.

The extent to which a gene is expressed ultimately depends on the frequency at which RNA polymerase initiates transcription from its promoter. Early studies focused on transcription factors that influence local RNA polymerase activity upon binding to specific nucleotide sequences in promoters and enhancers. However, the rate of transcription is also influenced by modifications to chromatin that can create differences in the local environment, dependent on cell type and stage of development (reviewed in 1). These modifications to DNA or histones that regulate transcription are collectively termed epigenetic, as they may allow heritable changes in gene expression without changes to the DNA sequence itself. Eukaryotic chromatin is organized into nucleosomes, in which genomic DNA is wrapped around histone proteins, separated by free DNA of varying length (2). Each nucleosome contains 147 base pairs of DNA and an octamer of proteins typically comprised of two subunits each of histones H2A, H2B, H3, and H4. Each histone contains a core that mediates interhistone interactions within the octamer as well as with DNA, and an extended tail. A diverse group of enzymes catalyze the addition or removal of covalent modifications to DNA or histones, primarily on the histone tail, as well as nucleosome remodeling. An association between certain chromatin modifications and the probability of transcription has been well established, such as H3K4 trimethylation (me3) at actively transcribed promoters and H3K27me3 with closed chromatin. Histone modifications influence the spacing of nucleosomes along DNA and their tendency to physically interact, creating variations in chromatin compaction throughout the genome. Relatively compact, or closed, chromatin (also termed B compartment) is unfavorable to transcription, while less compact, or open, chromatin (also termed A compartment) is more favorable. In addition, chromatin modifications alter the binding of transcriptional regulators that recognize modified nucleotides or amino acids. Chromatin modifications also regulate looping of DNA to position promoters near enhancers separated by long genomic distances (3). Whether a genomic locus is positioned in the transcriptionally repressive environment near the nuclear membrane or in a permissive environment in the interior is also critical for regulating gene expression (4).

The focus of this review is on DNA and histone modifications that regulate gene expression at key checkpoints in thymocyte development (**Figures 1** and **2**; **Table 1**). The most common modifications fall into four general categories: DNA methylation, histone methylation, histone acylation/acetylation, and histone ubiquitination. DNA methyltransferases (DNMTs) add methyl groups to cytosines preferentially in symmetrical CpG dinucleotide sequences, a mark typically associated with transcriptional repression. TET proteins may subsequently modify these methyl groups, allowing for either passive or active demethylation (5, 6) associated with transcriptional activation. Both the histone cores that form the nucleosome as well as the extended basic (lysine- and arginine-rich) tails undergo extensive modifications (7), with methylation and acetylation being extensively studied (2). Methylation is catalyzed by histone methyl transferase (HMT) enzymes and removed by histone demethylases (KDMs), while acetyl groups are added by histone acetyltransferase (HAT) enzymes and removed by histone deacetylase (HDAC) enzymes. Histone ubiquitination by polycomb repressive complex 1 (PRC1) is involved in gene repression (8). In

**Table 1 Summary of gene-deficient mice[a]**

| Modification | Gene | Model | Phenotype | Reference |
|---|---|---|---|---|
| DNA methylation | *DNMT1* | Lck-cre | DN to DP block | 33 |
| | *DNMT1* | CD4-cre | No effect | 33 |
| | *DNMT3a* | Mx1-cre | Increased ETP, DN2 | 32 |
| | *DNMT3b* | EμSR-tTA | No effect | 34 |
| | *DNMT3b* | ICF mutants | DN to DP block | 39 |
| 5mc oxidation/DNA demethylation | *TET2* | Vav-cre | No effect | 43 |
| | *TET2* | CD2-cre | No effect | 44 |
| | *TET3* | CD4-cre | No effect | 45 |
| | *TET2/TET3* | *TET2* KO/CD4-cre *TET3* cKO | Expanded NKT population | 45 |
| | *TET1/TET3* | RORC-cre | No effect | 46 |
| Histone acetylation | *CBP* | Lck-cre | Reduced DP, increased innate CD8 | 58, 60 |
| | *p300* | Lck-cre | Reduced DP | 58 |
| | *CBP/p300* | Lck-cre | Severely reduced DP | 58 |
| | *CBP/p300* | Foxp3-cre | Decreased Treg population | 61 |
| | *Mof* | Lck-cre | DN3/DN4 block | 62 |
| | *Kat7* | CD4-cre | NKT population lost, loss of peripheral T cells | 63 |
| | *GCN5* | Lck-cre | NKT population lost | 64 |
| Histone deacetylation | *HDAC1* | CD4-cre | No effect | 67 |
| | *HDAC1* | Lck-cre | No effect | 68 |
| | *HDAC2* | CD4-cre | No effect | 69 |
| | *HDAC2* | Lck-cre | No effect | 68 |
| | *HDAC1/HDAC2* | CD4-cre | SP lineage integrity defect | 69 |
| | *HDAC1/HDAC2* | Lck-cre | DN to DP block | 68 |
| | *HDAC3* | Lck-cre | Positive selection defect | 76 |
| | *HDAC3* | CD2-icre | DP, CD4 lineage defect, decreased positive selection | 77–79 |
| | *HDAC3* | CD4-cre | Loss of iNKT cells | 80 |
| | *HDAC4* | CD4-cre | No effect | 70 |
| | *HDAC5* | KO | No effect | 71 |
| | *HDAC6* | KO | No effect | 72 |
| | *HDAC7* | Lck-cre | Reduced cellularity, complex effects | 83 |
| | *HDAC11* | KO | No effect | 73 |
| | *Sirt1* | CD4-cre | No effect | 74 |
| | *Sirt3* | KO | No effect | 75 |
| | *Tcf1* | KO | Decreased ETP, T cell specification defect | 90, 91 |
| | *Tcf1* | CD4-cre | No effect | 87 |
| | *Lef1* | CD4-cre | No effect | 87 |
| | *Tcf1/Lef1* | CD4-cre | SP lineage integrity defect | 66, 87 |

(*Continued*)

**Table 1 (*Continued*)**

| Modification | Gene | Model | Phenotype | Reference |
|---|---|---|---|---|
| Histone methylation | *Ezh1* | Vav-cre | No effect | 107 |
| | *Ezh2* | Mx1-cre | DN to DP block | 108 |
| | *Ezh2* | CD4-cre | Expanded NKT population | 109 |
| | *KMT1A* | KO | No effect | 114 |
| | *KMT1C* | Lck-cre | No effect | 115 |
| | *KMT1E* | Lck-cre | Positive selection defect, loss of CD4 and CD8 SP | 116, 117 |
| Histone demethylation | *LSD1* | Vav-cre | No effect | 119 |
| | *JARID1b* | KO | No effect | 120 |
| | *UTX* | CD4-cre | No effect | 121 |
| | *JMJD3* | CD4-cre | No effect | 121 |
| | *UTX/JMJD3* | CD4-cre | Increased retention of SP, NKT population lost | 121, 123 |
| | *UTX/JMJD3* | Vav-cre | NKT population lost | 124 |
| Histone ubiquitinylation | *Ring1A* | KO | No effect | 126 |
| | *Ring1B* | Mx1-cre | No effect | 127 |
| | *Ring1A/Ring1B* | *Ring1A* KO/Lck-cre *Ring1B* cKO | DN to DP block, Failure to shut off B cell program | 128 |
| | *Ring1A/Ring1B* | *Ring1A* KO/CD4-cre *Ring1B* cKO | No effect | 128 |
| Histone deubiquitinylation | *USP16* | CD4-cre | No effect | 130 |
| | *USP21* | CD4-cre | No effect | 131 |
| | *MYSM1* | KO | Decreased ETP, DN2 | 133, 134 |
| | *Bap1* | Cre-ERT2 | DN3 arrest | 9 |
| | *Bap1* | CD4-cre | No effect | 9 |
| Histone arginine methylation | *CARM1* | KO | Decreased ETP, DN2 | 135, 136 |
| | *PRMT5* | CD4-cre | Loss of NKT population | 139 |

Abbreviations: cKO, conditional knockout; DN, double negative; DP, double positive; ETP, early thymic progenitor; iNKT, invariant NKT; KO, knockout; NKT, natural killer T cell; Treg, regulatory T cell.

[a]Mice are grouped according to the type of chromatin modification catalyzed by the targeted genes listed in the second column. The third column indicates that the mouse was either a germline knockout or a conditional knockout driven by the indicated cre transgene. Mice in which two genes were simultaneously targeted are also presented as indicated by the slash. "No effect" indicates the lack of a significant change in thymic cellularity or in the distribution of cells between the major conventional T cell subsets. See text for a detailed description of the phenotypes listed.

thymocytes, deubiquitination of H2AK119 is mediated by BAP1 (9). These modifications can either promote or impair transcription depending on the specific type and position of modification. It should be noted that these enzymes typically do not function alone but are often part of larger complexes that may contain more than one enzyme, thus linking chromatin marks that enhance or suppress transcription. In addition, a modification established by one enzyme can recruit additional protein complexes with additional enzymes to reinforce open or closed chromatin.

## OVERVIEW OF T CELL DEVELOPMENT

T cell development occurs in the thymus, beginning with the entry of uncommitted, multipotent progenitors that differentiate into various effector T cell lineages, including conventional CD4 helper T cells, conventional CD8 cytotoxic T cells, invariant natural killer (iNKT) cells, regulatory

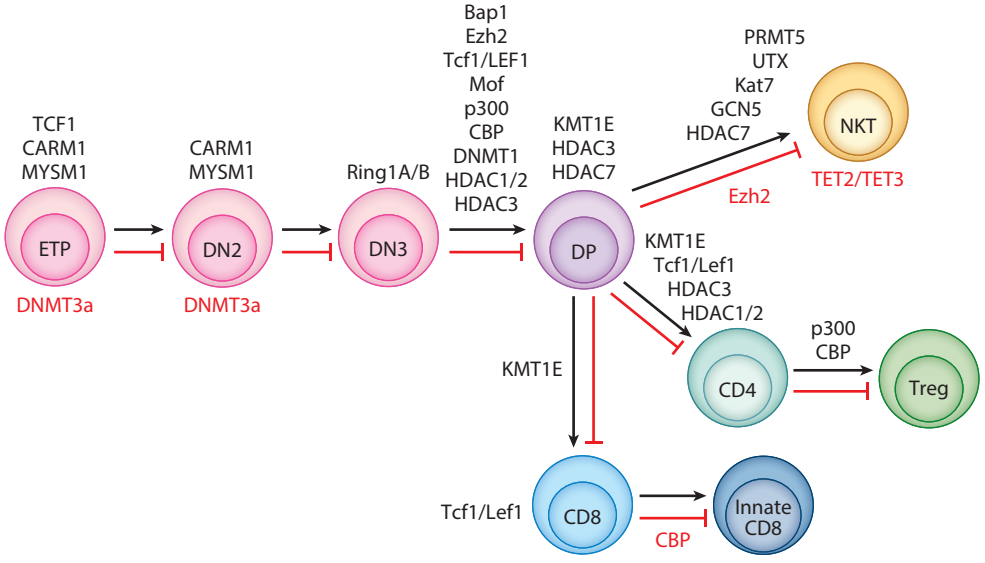
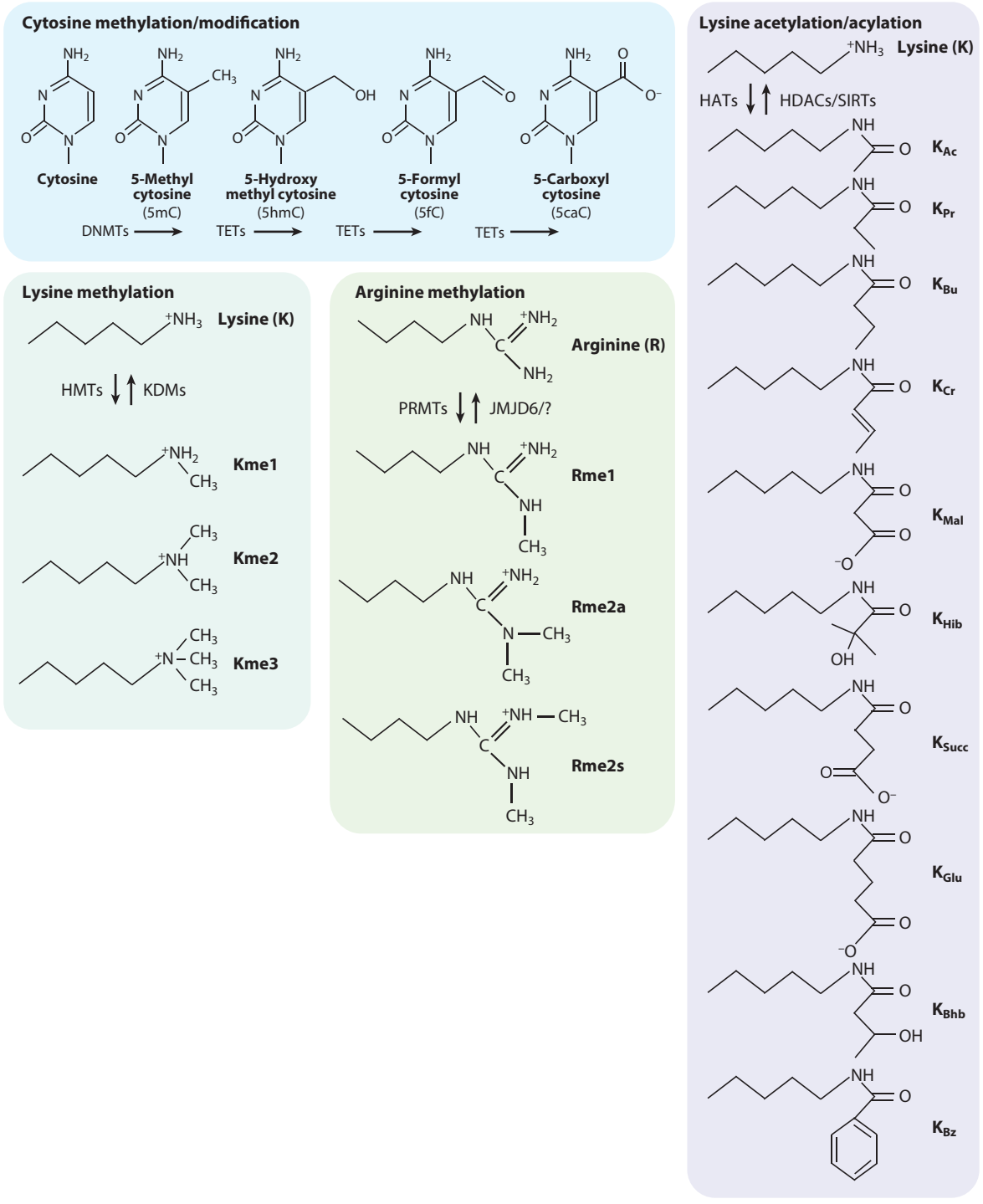



**Figure 1**

Chromatin-modifying enzymes in αβ T cell development. A schematic of αβ T cell development is shown, listing chromatin-modifying enzymes at the stage(s) in which they function. Black denotes positive regulation (i.e., a defect when deleted), while red denotes negative regulation (i.e., enhancement when deleted) during T cell development. In some cases, effects are observed only when two related genes are simultaneously deleted, which is denoted by a slash between the gene names. Please note that as analysis of DN4 and ISP cells was not consistently performed in all studies, a simplified scheme is presented in which the DN3 stage transitions to DP thymocytes. Abbreviations: DP, double positive; ETP, early thymic progenitor; ISP, immature single positive; NKT, natural killer T cell; Treg, regulatory T cell.

T cells, and γδ T cells (reviewed in 10–14). In mice, the earliest precursor in the thymus is the ETP (early thymic progenitor), which enters the thymus at the corticomedullary junction. The ability to differentiate into other hematopoietic lineages is soon lost, with irreversible commitment to the T cell lineage occurring at the DN2 stage, which requires the transcription factor Bcl11b. Subsequent bifurcation into the γδ or αβ lineage initiates rearrangement of their respective receptors. Successful rearrangement of the β chain at DN3, and successful pairing with pre-Tα, is required to generate a signal to transit through the β-selection checkpoint for transition to the double-positive (DP) stage. At the DP stage, TCRα is rearranged, leading to testing of the TCR for signal strength. DP thymocytes with a canonical, invariant $V_{\alpha}14\text{-}J_{\alpha}18$ rearrangement, with a limited $V_{\beta}$ pairing, are positively selected into the iNKT cell lineage at the DP stage. Most DP thymocytes will fail to generate a signal of sufficient strength, which leads to death by neglect. Strong signals stemming from the recognition of self-antigens in self-MHC lead to negative selection and their removal. Weaker signals allow for positive selection and testing of coreceptor specificity for correct commitment to the CD4 or CD8 lineage. Further testing of TCR specificity by peripheral tissue antigens expressed by thymic epithelial cells (TECs) allows for further negative selection to remove autoreactive T cells prior to egress. However, some TCRs with stronger signals generate $Foxp3^{+}$ regulatory T cells (Tregs), to suppress escapee autoreactive T cells in the periphery.

As thymocytes successfully transition through the checkpoints of T cell development, there are dynamic changes in gene expression (15, 16). The process of T cell specification leads to gradual




(*Caption appears on following page*)

**Figure 2** (*Figure appears on preceding page*)

DNA and histone modifications: Selected modifications of DNA and histone modifications on lysine and arginine. Only the side chains of lysine and arginine are shown. Cytosines are modified by members of the DNMT and TET families. Lysine methylation is mediated by HMTs and KDMs. Lysine acetylation and acylation are mediated by HATs, HDACs and SIRTs. Arginine methylation is mediated by PRMTs, while the enzymes responsible for arginine demethylation are not conclusively identified but may include JMJD6 (represented by JMJD6/?). Abbreviations: DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; $K_{Ac}$, acetylated lysine; $K_{Bhb}$, β-hydroxybutyrylated lysine; $K_{Bu}$, butyrylated lysine; $K_{Bz}$, benzoylated lysine; $K_{Cr}$, crotonylated lysine; KDM, lysine demethylase; $K_{Glu}$, glutarylated lysine; $K_{Hib}$, 2-hydroxyisobutyrylated lysine; $K_{Mal}$, malonylated lysine; Kme1, monomethylated lysine; Kme2, dimethylated lysine; Kme3, trimethylated lysine; $K_{Pr}$, propionylated lysine; $K_{Succ}$, succinylated lysine; PRMT, protein arginine methyltransferase; Rme1, monomethylated arginine; Rme2a, asymmetric dimethylated arginine; Rme2s, symmetric dimethylated arginine; SIRT, sirtuin; TET, ten-eleven translocation.

changes in gene expression, although a shift occurs with commitment from DN2a to DN2b (15, 16). In contrast, there are substantial shifts at DN3a and DP stages, corresponding to β-selection and positive-selection checkpoints (15, 16). In comparison, only minor changes were observed between CD4 and CD8 single-positive (SP) thymocytes (15). In general, these changes in gene expression correlate with alterations in DNA methylation and histone modifications, although there are exceptions, which are described in greater detail below.

## DYNAMIC CHANGES IN DNA METHYLATION DURING T CELL DEVELOPMENT

The most prevalent DNA modification is methylation of cytosine to generate 5-methylcytosine (5mC), which occurs predominantly on both strands at palindromic CpG dinucleotide sequences and is mediated by the DNMT family of DNA methyltransferases (1, 17, 18). In general, CpG methylation corresponds with gene silencing, such as during X chromosome inactivation (19) and inactivation of endogenous retroviruses (20). Analysis of genome-wide changes in cytosine methylation found extensive changes as multipotent progenitors differentiated into the myeloid, lymphoid, or T cell lineage (21). However, this initial examination used methods that did not discriminate between cytosine modifications, as 5mC can undergo additional modification by oxidation (5). TET proteins catalyze the oxidation of 5mC in DNA to 5-hydroxymethylcytosine (5hmC), which may be further oxidized to 5-carboxylcytosine (5caC) and 5-formylcytosine (5fC), which are the first steps toward removal of the 5mC mark, as no enzyme that directly converts 5mC back to cytosine has been identified (6, 22). In proliferating cells, oxidation of 5mC to 5hmC blocks maintenance methylation by DNMT1, thus diluting this mark and leading to passive demethylation (23). 5fC and 5caC are recognized and removed by thymine DNA glycosylase, which triggers base excision repair for replacement with unmodified cytosine in the absence of DNA replication, termed active demethylation (24–26). Interestingly, while 5mC is associated with gene repression, 5hmC is associated with gene expression. Genome-wide mapping of 5hmC marks during T cell development revealed a strong enrichment of 5hmC marks in highly expressed genes, and they were found to coincide with RNA polymerase II occupancy, H3K36me3 across the gene body, and H3K4me3 at the transcriptional start site (27, 28). 5hmC enrichment occurred primarily in gene bodies, though there was also enrichment at active tissue-specific enhancers and associated with H3K4me1 and H3K27Ac (27, 28). Stage-specific increases in 5hmC were also observed in the genes for several key transcription factors for T cell development, including ThPOK in CD4 SP thymocytes and Runx3 in CD8 SP thymocytes. Therefore, there is dynamic regulation of cytosine methylation and hydroxymethylation during development that corresponds with stage-specific gene expression.

## ADDITION AND MAINTENANCE OF CPG CYTOSINE METHYLATION

Conversion of cytosines in CpG sequences to 5mC is mediated by the DNMT family of enzymes, which includes DNMT1, DNMT3a, and DNMT3b in mammals (18) (**Figure 2**). Cytosine methylation at CpG dinucleotides occurs on both DNA strands. In general, DNMT1 functions to maintain CpG methylation after DNA replication on the newly synthesized strand. In contrast, DNMT3a and DNMT3b function as de novo DNA methylases at previously unmethylated sites. Knockout mice targeting the genes for each DNMT have been generated, resulting in severe developmental abnormalities and either embryonic or rapid perinatal lethality in each case (29, 30). In thymocytes, DNMT1 is expressed throughout development, while expression of DNMT3a and DNMT3b is dynamic. DNMT3b is expressed at double-negative (DN) and SP stages, while DNMT3a has two splice isoforms with different expression patterns during T cell development (31, 32). DNMT3a1 is expressed starting at the DP stage while the DNMT3a2 isoform is expressed throughout T cell development. Functional differences between DNMT3a splice isoforms in T cell development are not understood. Consistent with its role in maintaining CpG methylation after DNA replication, DNMT1 is required at the proliferative burst induced by successful β-selection but is not needed after the DP stage to complete T cell development (33). Without DNMT1 in early T cell development, there is increased apoptosis and few DP thymocytes (33). However, thymocyte development is minimally impeded by loss of DNMT3a or DNMT3b alone, with a twofold increase in ETP and DN2 stages in the absence of DNMT3a due to decreased apoptosis (32, 34). Dual deficiency of DNMT3a and DNMT3b in hematopoietic stem cells did not alter their ability to reconstitute irradiated recipients or their ability to produce peripheral T cells at the expected frequency (35). However, a detailed analysis of peripheral T cell populations or thymocyte development was not performed (35). While there was no effect on thymocyte development when DNMT3b was deleted, point mutations in DNMT3b cause ICF syndrome, a rare disease characterized by immunodeficiency and a reduced number of T cells (36–38). Knock-in mice with DNMT3b mutations replicating the patient mutations are runted and die within four weeks with a tenfold decrease in thymic cellularity and a block at the DN stage (39). DNMT3a knockout mice have a similar phenotype, with runting, decreased thymocyte numbers, and lethality within four weeks of birth (30). This discrepancy may be due to changes in function of DNMT3b with the ICF point mutations as compared to complete loss. Alternatively, the loss of DNMT3a or ICF mutations in DNMT3b may exert non-cell-intrinsic effects on thymocyte development, which will need to be tested such as through the use of bone marrow chimeras.

## MODIFICATION AND REMOVAL OF CYTOSINE METHYLATION

No enzyme has been identified that directly converts 5mC to cytosine. The TET family of dioxygenases catalyze the oxidation of 5mC in DNA to 5hmC, 5caC, and 5fC (**Figure 2**). Thymine DNA glycosylase recognizes and removes 5fC and 5caC, leading to base excision repair for replacement with unmodified cytosine in the absence of DNA replication (24–26). It is unclear whether conversion of methylated to unmodified cytosine is always the physiologically relevant end result of TET activity, or whether 5hmC is read separately as an epigenetic mark (6, 22, 40). Within the TET family, TET2 and TET3 are expressed at higher levels than TET1 in thymocytes (41). TET3 deficiency causes embryonic or perinatal lethality, while TET1 or TET2 knockout mice are grossly normal and fertile (22, 41, 42), although loss of TET2 enhanced hematopoietic stem cell reconstitution upon serial transplants (41, 43). No alterations in thymic or peripheral T cells were observed in vav-cre TET2 conditional knockout (cKO) mice (43), CD2-cre TET2 cKO mice (44), or CD4-cre TET3 cKO mice (45). As there is likely

redundancy in TET function, conditional knockouts for two family members have been generated. Combined deficiency in TET1/TET3 (using RORC-cre) did not alter thymocyte development (46). However, the concurrent loss of TET2/TET3 led to uncontrolled expansion of iNKT cells (45) or defects in Treg stability, leading to lethal autoimmunity (47). Global 5hmC content was significantly reduced, but not eliminated, in DP and SP thymocytes in CD4-cre TET2/TET3 double-knockout (dKO) mice, demonstrating that TET2 and TET3 were responsible for the majority of 5hmC in thymocytes. However, no alteration in conventional T cell development was observed (45). It may be that a single TET family member is sufficient for conventional T cell development, as all three are coexpressed. However, no study has yet utilized cKO mice deficient for all TET family members, or produced thymocytes that lack all 5hmC. Alternatively, TET family members may have a greater role earlier in T cell development, as both examinations of TET2/TET3 and TET1/TET3 cKO mice used cre's leading to deletion at the DP stage, while deletion at the DN stage has yet to be explored.

## DYNAMIC CHANGES IN HISTONE ACETYLATION AND METHYLATION DURING T CELL DEVELOPMENT

Histones are subject to at least ten types of posttranslational modifications on more than 60 amino acids among the four core histones, primarily on their extended histone tails (**Figure 2**). Together, these posttranslational modifications comprise the histone code. In addition to the type of modification, there is diversity, as lysine can be mono-, di-, or trimethylated. Arginines can be either mono- or dimethylated, and the dimethylation of arginines can be either symmetric or asymmetric. There have been several excellent reviews on posttranslational histone modifications (2, 48–52), and therefore these will not be reviewed extensively here. It should also be emphasized that some sites can be modified in more than one way (e.g., either methylation or acetylation), and thus one mark must be removed prior to addition of an alternative one. The generation of antibodies for ChIP-seq (chromatin immunoprecipitation sequencing) that recognize site-specific methylation or acetylation modifications was critical to understanding the role of these changes in promoting or inhibiting transcription. The initial groundbreaking work to understand histone changes during T cell development analyzed five thymic populations to follow changes over time (DN1, DN2a, DN2b, DN3, and DP) (16). While expressed genes displayed a high level of H3Ac and H3K4me2 modifications and a very low level of H3K27me3 modification, nonexpressed (or silent) genes did not follow a simple pattern. Approximately half the silent genes lacked H3Ac, H3K4me2, and H3K27me3, and only a small subset of silent genes were marked by H3K27me3 alone. Some silent genes possessed both H3K27me3 and H3K4me2 modifications; these were generally either newly repressed, indicated by expression in the previous stage, or poised for upregulation at the next stage. Thus, multiple mechanisms may lead to gene silencing that does not simply correlate with the presence of H3K27me3. Similar dual marking with both H3K27me3 (repressive) and H3K4me3 (associated with active promoters) was also observed at the CD8 locus at the DN stage prior to CD8 expression at the DP stage (53). The H3K27me3 mark is removed in DP and CD8 SP thymocytes, while CD4 SP thymocytes downregulate H3K4me3 and reestablish H3K27me3. Similar dynamic histone modifications were also observed at the ThPOK gene, which is required for generation of CD4 SP thymocytes and is induced concurrently with commitment to the CD4 lineage (54–56). Both H3K4me3 and H3K27me3 modifications were present in the Thpok promoter in DN and DP cells. Commitment to the CD8 lineage induced removal of the positive H3K4me3 mark, while commitment to the CD4 lineage and expression of ThPOK induced removal of the negative H3K27me3 mark.

## HISTONE ACETYLTRANSFERASES IN T CELL DEVELOPMENT

There are 17 members of the HAT family, all of which are coexpressed during T cell development (**http://immgen.org**). These are broadly divided into four families: the GNAT family (HAT1, GCN5, PCAF, and ATF2), CBP/p300 family, MYST family (Tip60, MOZ, MORF, HBO1, and MOF), and SRC family (NCOA1, NCOA2, and NCOA3); three do not neatly fall into the other groups (Clock, Taf1, TFIIIC90) (57). The GNAT, CBP/p300, MYST, and SRC families share considerable homology and domain structure. Each contains domains for protein-protein interactions, including reader domains that recognize specific chromatin modifications, allowing for recruitment to DNA. They serve nonredundant functions, as many individual HAT knockouts in mice lead to embryonic or early lethality and have different preferences for site-specific modifications. The specific role of individual HATs in T cell development is largely unknown, as few T cell–specific knockouts have been described. Conditional ablation of either CBP or p300 using Lck-cre leads to decreased DP thymocyte numbers, while dual deletion of CBP/p300 synergizes to reduce the number of DP thymocytes by tenfold with very few peripheral T cells (58). The target genes responsible for this dramatic block in conventional T cell development in the CBP/p300 dKO have not yet been identified. However, one direct target of p300 is CCR9, which regulates intrathymic thymocyte migration (59). Interestingly, although in general the number of SP thymocytes was similarly decreased in the absence of CBP or p300, the number of CD8 SP thymocytes was not decreased in Lck-cre CBP cKO mice (58). Rather, the loss of CBP drives development of innate CD8 T cells (60). A similar synergy is observed in the Treg lineage, where combined deletion of CBP/p300 using Foxp3-cre leads to a decrease in the absolute numbers of Tregs in the thymus and severe autoimmunity similar to the case of Foxp3 mutant mice (61). This is due in part to direct effects on Foxp3 expression, as both CBP and p300 associate with the Foxp3 locus (61). Conditional deletion of *Mof* using Lck-cre led to a severe decrease in thymic cellularity with a block at DN3/DN4 (62). Total H4K16Ac was decreased in the absence of *Mof*, indicating that it is the primary acetyltransferase for this modification during T cell development.

Both KAT7 and GCN5 are critical for the development of iNKT cells. Conditional deletion of KAT7 (also known as HBO1 or Myst2) using CD4-cre had a minimal effect on conventional T cell development but almost completely abrogated iNKT cell development, although the mechanism is not known (63). The loss of KAT7 severely decreased total H3K14Ac in DP and SP thymocytes, demonstrating that KAT7 is the primary HAT responsible for this chromatin mark during T cell development. Within a competitive environment in a mixed bone marrow transplant, however, KAT7-deficient thymocytes could not compete with wild-type thymocytes. Although thymic T cell development was normal, there were few CD4 or CD8 T cells in the periphery in the absence of KAT7. This was not due to a defect in thymic maturation but rather due to decreased cell survival altering peripheral T cell persistence. Conditional deletion of GCN5 also led to only a small (~20%) reduction in thymocyte numbers and conventional T cell development, but iNKT development was blocked (64). The primary function of GCN5 in iNKT cells is not related to histone modification but rather to regulation of Egr2 function by direct acetylation.

## HISTONE DEACETYLASES IN T CELL DEVELOPMENT

At least 18 HDACs exist in mammals, and they are divided into four classes: class 1 is comprised of HDAC1, 2, 3, and 8; class 2 is comprised of HDAC4, 5, 6, 7, 9, and 10; class 3 is comprised of the Sirtuin (Sirt) family of enzymes SIRT1, 2, 3, 4, 5, 6, and 7; finally, HDAC11 is the sole member of class 4 (65). Several HDACs are found in multiprotein complexes along with other chromatin-modifying enzymes, which may be recruited to DNA by transcription factors. According to the

Immunological Genome Project Database (**http://immgen.org**), all 18 of these HDACs are expressed during thymocyte development. In addition, two transcription factors that are required for normal development and histone acetylation in T cells were recently shown to have intrinsic HDAC activity as well (66), thus expanding HDACs beyond the conventional set. Mice have been generated with individual deficiency in HDAC1 (67, 68), HDAC2 (68, 69), HDAC4 (70), HDAC5 (71), HDAC6 (72), HDAC11 (73), Sirt1 (74), or Sirt3 (75), but there was no effect on thymic development.

Unlike the other HDACs that have been analyzed, HDAC3 has been found to have a unique and essential role at several stages in T cell development. HDAC3 deletion early in T cell development using Lck-cre (76) or CD2-icre (77) blocked positive selection. Although the expression of many genes is dysregulated in DP thymocytes when HDAC3 is absent (76, 77), the block in positive selection is specifically due to an inability to downregulate RORγt (77). When the positive selection block is bypassed by RORγt deficiency, however, only CD8 SP thymocytes are produced. MHC class II–restricted OT-II transgenic TCRs, which normally produce CD4 SP thymocytes, are diverted into the CD8 lineage. HDAC3-deficient DP thymocytes aberrantly express CD8 lineage genes, including Runx3 and Patz1, due in part to increased H3K9Ac and H3K27Ac at these genes leading to the formation of superenhancers (78). Thus, HDAC3 is required to repress the CD8 lineage program to allow development of the CD4 lineage (78). HDAC3 is also required to repress expression of the purinergic ATP receptor P2RX7 (79). Inappropriate expression of P2RX7 in DP thymocytes contributes to increased cell death (79). HDAC3 is also required for generation of iNKT cells (80), and for post–positive selection T cell maturation (81). Thus, HDAC3 plays a critical role at many stages of T cell development, which cannot be compensated for by the expression of the other 17 members of the HDAC family.

HDAC7 also has an important role during T cell development. Lck-cre HDAC7 cKO mice have partial but significant reductions in the number of DP and both CD4 and CD8 SP cells. HDAC7 cKO thymocytes became apoptotic more rapidly than wild-type thymocytes in ex vivo cultures, which was due to upregulation of Nur77 expression. Interestingly, TCR engagement triggers the export of HDAC7 from the nucleus (82). During T cell development HDAC7 is predominantly nuclear in DP thymocytes, but predominantly cytoplasmic in SP thymocytes (83). This indicates that HDAC7 activity during T cell development may be regulated by TCR signaling. To understand the function of TCR-mediated HDAC7 export during T cell development, thymocyte-specific transgenic mice with an HDAC7 mutation (HDAC7-ΔP) that is resistant to TCR-stimulated nuclear export were generated (84). By six months of age, these mice developed lethal, multi-organ autoimmune disease. Gene expression profiling demonstrated altered expression of many genes involved in negative selection, indicating that altered negative selection contributes to the development of autoimmune disease. In addition, there are defects in generation of iNKT cells in both HDAC7-ΔP and HDAC7 cKO mice, which are mediated through HDAC7 regulation of PLZF function (85). Interestingly, restoration of iNKTs partially ameliorated autoimmune disease in HDAC7-ΔP mice, demonstrating that altered negative selection of conventional T cells is not the only mechanism involved.

While single deletion of HDAC1 (67, 68) or HDAC2 (68, 69) did not disrupt T cell development, these proteins function redundantly, as combined deletion of HDAC1 and HDAC2 had dramatic effects (68, 69). Thymocytes from Lck-cre HDAC1/HDAC2 dKO mice had a severe block in T cell development (68). Thymic cellularity was reduced fivefold, with blocks at both the DN3 to DP transition and positive selection. In addition, reduced commitment to the CD4 lineage was observed. H3K9Ac was strongly increased in the dKO, demonstrating an important role for HDAC1 and HDAC2 in deacetylating this chromatin mark in thymocytes. Analysis of altered gene expression found that several genes involved in TCR signaling were downregulated, which

likely contributed to the block in positive selection. HDAC1 and HDAC2 were previously shown to regulate the DNA damage response through deacetylation of H3K56Ac and H4K16Ac in non-immune cells (86). HDAC1 and HDAC2 are also important for regulating genomic stability in thymocytes, as HDAC1/2 dKO mice had increased γ-H2AX staining, indicative of DNA damage, and H3K56Ac and K4K16Ac were also increased. Deletion of HDAC1/2 later in T cell development using CD4-cre disrupted lineage commitment, with the production of mature $CD4^+CD8^+$ thymocytes that persist into the periphery (69). These mature $CD4^+CD8^+$ thymocytes were MHC class II restricted and expressed ThPOK. However, stimulation of $CD4^+$ T cells induced expression of CD8, Runx3, and genes associated with CD8 cytotoxic effector function. These results collectively show that HDAC1 and HDAC2 have overlapping functions but are collectively essential for genome integrity and TCR signaling, and also act later in development to maintain the integrity of the CD4 lineage by repressing genes associated with CD8 effector function.

Although they do not fall into any of the four classes of conventional HDACs, the transcription factors Tcf1 and Lef1 have also been found to possess intrinsic HDAC activity (66). Although individual deletion using CD4-cre did not cause a significant phenotype, dual loss of Lef1/Tcf1 decreased CD4 SP generation, in part due to a block in ThPOK expression (87). In addition, CD4 was aberrantly expressed in CD8 SP thymocytes, along with other genes characteristic of the CD4 lineage (66, 87). Analysis of chromatin modification of these CD4 lineage genes in Lef1/Tcf1 dKO thymocytes revealed a substantial increase in histone acetylation relative to wild-type CD8 SP cells. Increases in histone acetylation occurred at over 7,000 Tcf1 binding sites identified by ChIP-seq analysis. In vitro translated Tcf1 catalyzed deacetylation of purified histones and acetylated lysine substrates, demonstrating its role as a histone deacetylase. With the use of site-directed mutagenesis, a 30–amino acid segment of Tcf1 required for its HDAC activity was identified that is conserved in Lef1 and also bears some homology to conventional HDACs. Wild-type Tcf1 was introduced into Tcf1/Lef1 cKO mice by retroviral transduction, which resulted in a significant decrease in histone acetylation at CD4 lineage genes in $CD8^+$ T cells. In contrast, expression of a Tcf1 mutant with substitutions at residues critical for HDAC activity did not cause a change in histone acetylation. Cumulatively, these results suggest that Tcf1 and Lef1 are critical for establishing lineage identity by, at least in part, silencing expression of lineage-specific genes directly by deacetylation. This also suggests a novel mechanism for site-specific histone modification mediated directly by a transcription factor without an associated protein complex. Tcf1 and Lef1 have also been shown to play important roles earlier in T cell development, but it is not known whether their deacetylase activities are required at these other stages. The block at the CD8 immature single-positive stage is exacerbated in Tcf1/Lef1 knockout mice, as compared to loss of Tcf1 alone (88, 89). Tcf1 is critical for T cell specification at the ETP stage (90, 91). Interestingly, Tcf1 plays an important role in generating open chromatin at T lineage genes during T cell development (92). Ectopic Tcf1 expression also drove chromatin opening at T lineage genes in fibroblasts (92). It is not known whether intrinsic HDAC activity of Tcf1 is required for induction of chromatin accessibility of T lineage genes.

## HATs AND HDACs MEDIATE MODIFICATIONS BEYOND HISTONE ACETYLATION

While the focus above is on histone acetylation mediated by HATs and HDACs, these enzymes also can acetylate nonhistone targets and mediate addition or removal of numerous acylations in addition to acetylation (**Figure 2**). For example, the activity of the transcription factor Foxp3 in Tregs is regulated by acetylation by p300 and deacetylation by Sirt1 (93). In addition, selected HATs and HDACs have the ability to modify histones by acylations other than acetylation. Mass

spectrometry has demonstrated that histones in vivo undergo a variety of acylations at lysine residues: propionylation ($K_{Pr}$), butyrylation ($K_{Bu}$), crotonylation ($K_{Cr}$), succinylation ($K_{Succ}$), malonylation ($K_{Mal}$), 2-hydroxyisobutyrylation ($K_{Hib}$), glutarylation ($K_{Glu}$), β-hydroxybutyrylation ($K_{Bhb}$), and benzoylation ($K_{Bz}$) (reviewed in 94, 95). These acylations can substantially alter the hydrophobicity or charge at that lysine position. $K_{Pr}$, $K_{Bu}$, $K_{Cr}$, and $K_{Bz}$ are hydrophobic. $K_{Succ}$, $K_{Mal}$ and $K_{Glu}$ are acidic, thus switching the positive charge of lysine to a negative charge. $K_{Hib}$ and $K_{Bhb}$ are polar, which potentially permits hydrogen bonds with molecules in close proximity. Changes in acylation also change the recruitment of readers to these sites. While proteins with bromodomains bind to (read) acetylated lysines, not all bromodomains can recognize alternative acylations (96). Similarly, not all HATs or HDACs are able to add or subtract alternative acylations. For example, GCN5 can mediate $K_{Succ}$ but not $K_{Bu}$ or $K_{Cr}$ (97), while p300 has wider capabilities to mediate $K_{Pr}$, $K_{Bu}$, $K_{Cr}$, $K_{Glu}$, $K_{Hib}$, and $K_{Bhb}$ (98–102). It is not known whether the functions of GCN5 or p300 in T cell development involve histone acetylation or another acylation. Recently, mutations in p300 and CBP were identified that abrogate their function as acetyltransferases but not the ability to mediate crotonylation (103). These mutants were capable of activating transcription independent of the ability to acetylate histones, demonstrating that crotonylation also can lead to transcriptional activation (103). The roles of acylations other than acetylation during T cell development have yet to be explored. The possibility of alterations in these additional modifications should also be considered in interpreting the phenotypes of HAT and HDAC cKO mice.

## METABOLIC CHANGES MAY DRIVE HISTONE ACYLATIONS

It has been long recognized that metabolites directly impact epigenetic changes, such as *S*-adenosylmethionine (SAM) serving as the donor for DNMT-mediated methylation or α-ketoglutarate serving as a cofactor for TET enzymes (reviewed in 104). Recent work indicates that the relative availability of acyl-CoA metabolites directly influences histone lysine acylation (48). Acetyl-CoA is produced from glucose through the TCA cycle. Under starvation conditions where glucose is limiting, fatty acid oxidation predominates, and the acetyl-CoA that is produced is converted into ketone bodies that include β-hydroxybutyrate. β-Hydroxybutyrate is the substrate for generation of β-hydroxybutyryl-CoA for histone $K_{Bhb}$. Exogenous addition of β-hydroxybutyrate increased histone $K_{Bhb}$ and increased expression of genes associated with the response to starvation (105). Similarly, levels of histone $K_{Cr}$ are regulated by the levels of crotonyl-CoA (98). In the nucleus, the HAT GCN5 associates with the α-ketoglutarate dehydrogenase (α-KGDH) complex that catalyzes generation of succinyl-CoA from α-ketoglutarate, increasing local availability for histone $K_{Succ}$ (97). Thus, the relative availability of metabolites can alter histone marks and gene expression.

## HISTONE METHYLATION

In humans, there are 33 known histone lysine methyltransferases (HMTs), with each family member having high specificity for the histone residue that is methylated and the type of methylation (mono-, di-, or trimethylation) that it confers (**Figure 2**) (reviewed in 51). One of the best characterized histone methylations is H3K27me3, which is associated with transcriptional repression and is generated by the polycomb repressive complex 2 (PRC2). PRC2 complexes catalyze H3K27me2 and H3K27me3 modifications and minimally include either an Ezh1 or Ezh2 HMT enzyme along with EED and SUZ12 subunits (reviewed in 106). Ezh1 is not required for T cell development (107). However, Ezh2 is essential for early T cell development, as its deletion in hematopoietic stem cells led to a block at DN3 and a severe reduction in thymic cellularity (108). H3K27me3

was ablated in Ezh2-deficient DN thymocytes. However, it is not clear whether H3K27me3 dysregulation is responsible for the block. Ezh2 and other PRC2 components were found to localize to the cytoplasm of T cells in a complex also containing the signaling protein Vav1. Ezh2 methylated Vav1 in vitro and Ezh2-deficient T cells displayed a deficiency in TCR-mediated actin polymerization. Thus, Ezh2 may be required for pre-TCR signaling as well as H3K27me3 in DN3 thymocytes (108). While Ezh2 is expendable for conventional T cell development after the DP stage (108), iNKT cells were expanded tenfold in CD4-cre Ezh2 cKO mice (109). No differences in total H3K27me3 were observed in Ezh2-deficient iNKT cells. However, expression of PLZF, the lineage-defining factor for iNKT cells, was increased. Ezh2 directly methylates PLZF leading to its ubiquitin-mediated degradation. When Ezh2 is absent, PLZF is stabilized, disrupting iNKT cell homeostasis and increasing iNKT numbers.

High levels of H3K4me3 modifications are associated with promoters for genes that are actively transcribed. In mammals, the six known enzymes that catalyze H3K4 methylation are MLL1, MLL2, MLL3, MLL4, SETD1A, and SETD1B (reviewed in 110). MLL1/2 primarily generate H3K4me1 and H3K4me2 and MLL3/4 primarily generate H3K4me1, while SETD1A/B generate H3K4me3 (111). While SETD1A/B combined deficiency has not been generated in T cells, their essential role was demonstrated in mice deficient in CXXC1 (112). CXXC1 interacts with SETD1A/B and has two domains that can target the complex to DNA: by association with unmethylated CpG sequences through its N-terminal CXXC domain and association with H3K4me1-marked histones through its PHD domain (113). T cell specific deletion of CXXC1 using CD2-icre led to a severe block in T cell development with decreased thymocyte survival and a block in positive selection (112). There was a global disruption in H3K4me3, indicating that CXXC1 and its associated SETD1A/B complexes are the primary mediator of H3K4me3 in thymocytes. Thymic cellularity was dramatically decreased, due to disruption of RORγt expression at the DP stage, leading to decreased Bcl-xl expression (112). Cellularity in CD2-icre CXXC1 cKO mice was partially restored by expression of a Bcl2 transgene. Epigenetic alterations at the *RORC* gene extended beyond H3K4me3, and decreases in H3K9Ac and H3K27Ac were also observed, indicating a dynamic interplay between histone methylation and acetylation (112). The loss of CXXC1 also decreased expression of several genes critical for TCR signaling such as *Lck* and *ZAP-70*. Thus, transcriptional activation of genes essential for T cell development requires the targeting of SETD1A/B to their promoters.

H3K9 methylation is catalyzed by six known HMT enzymes (KMT1A through KMT1F) and is associated with transcriptional silencing. Neither KMT1A (also known as Suv39h1) nor KMT1C (also known as G9a) is required for thymocyte development (114, 115). However, KMT1E (also known as SetDB1 or ESET) is required for positive selection and the production of CD4 and CD8 SP thymocytes (116, 117). FcγRIIb is overexpressed in KMT1E-deficient thymocytes, and thymocyte development was partially restored in mice doubly deficient in KMT1E and FcγRIIb (117). Increased apoptosis in KMT1E thymocytes, in particular CD8 SP thymocytes, was also observed (116, 117). KMT1E mediates H3K9 trimethylation, and 24% of loci in DP thymocytes lost the H3K9me3 mark when KMT1E was deleted (117). Coexpression of the closely related enzyme KMT1F (SETDB2) may be responsible for the majority of thymic H3K9me3 when KMT1E is deleted, but this mechanism has yet to be tested.

## HISTONE DEMETHYLATION

There are 21 lysine demethylases that have been identified (51), broadly divided into FAD-dependent amine oxidases and 2-oxoglutarate-dependent oxygenases (reviewed in 51; 118). The

functions of many of these enzymes in T cell development are not yet known. Individual deletion of LSD1 (KDM1A), JARID1b (KDM5B), UTX (KDM6A), or JMJD3 (KDM6B) did not alter conventional T cell development (119–121). UTX and JMJD3 demethylate H3K27me3, which is associated with gene silencing (reviewed in 122). UTX and JMJD3 have overlapping functions in T cell development, which was elucidated when both were deleted using CD4-cre (121). In SP thymocytes, UTX and JMJD3 mediate removal of H3K27me3 at the *S1pr1* gene, which is required for thymic egress. In their absence, increased numbers of CD4 and CD8 SP thymocytes are retained in the thymus. The combined loss of UTX/JMJD3 resulted in alteration in H3K27me3 at less than 1% of genes, demonstrating specificity for demethylation. H3K27me3 removal at ThPOK, which is required for CD4 lineage commitment, was only modestly affected, indicating that additional lysine demethylases may also have an important, but as yet undefined, role in T cell development (121). However, combined deletion of UTX/JMJD3 had severe effects on iNKT cell development, which was primarily due to deficiency in UTX rather than JMJD3 (123, 124). iNKT cell development was partially restored when Ezh2, which mediates H3K27me3, was ablated concurrently with UTX (123). Thus, key genes for the iNKT cell lineage are directly controlled through regulation of H3K27 trimethylation by UTX and Ezh2.

## HISTONE UBIQUITINATION AND DEUBIQUITINATION

H3K27me3 modifications catalyzed by Ezh1 and Ezh2 (PRC2 enzymatic subunits) trigger recruitment of PRC1 dependent on chromodomain containing CBX subunits which bind methylated histones. PRC1 catalyzes the monoubiquitylation of histone H2A (H2AUb), primarily at lysine 119 (H2AK119Ub), and contains either a Ring1A or Ring1B E3 ubiquitin ligase (reviewed in 125). However, there are also PRC1 complexes lacking CBX proteins that are recruited to chromatin by other subunits independent of H3K27 methylation. Once associated with a genomic location, PRC1 promotes chromatin compaction through multiple mechanisms in addition to H2AUb modifications to nucleosomes. Ring1A and Ring1B have redundant functions in T cell development (126, 127), although deletion of both enzymes demonstrates that they are collectively essential (128). To generate mice without Ring1A or Ring1B in the T cell lineage, Ring1A KO mice were interbred with Lck-cre Ring1B cKO mice. In these Ring1A/Ring1B dKO mice, thymic cellularity was reduced by 95%, TCR$\beta^+$ cells were nearly absent, and T cell development was blocked at the DN3 stage. When CD4-cre was used instead of Lck-cre to generate dKO mice, no defects in T cell development occurred. *Cdkn2a* (encoding p16Ink4a and p19ARF) is a direct target of PRC1 complexes (127); however, deletion of *Ckdn2a* in the context of a Ring1A/Ring1B dKO did not restore thymic cellularity, although a few DP cells were produced. Interestingly, these DP thymocytes expressed CD19, produced B cells after adoptive transfer, and expressed a B-lineage gene program. When Pax5, a critical lineage-determining factor for B cells, was deleted in the context of a Ring1A/Ring1B dKO, thymocyte development was completely restored. Thus, Ring1A/Ring1B are required early in T cell development to shut off the B-lineage program.

H2AK119Ub can be removed by deubiquitinating enzymes, including USP16, USP21, MYSM1, and BAP1 (reviewed in 129). T cell development does not require USP16 [CD4-cre cKO mice (130)] or USP21 (131). MYSM1 is required at the earliest stages of T cell development, but this is primarily by regulating p53 protein expression at the posttranslational level rather than H2AK119 deubiquitination (132–134). Rather, BAP1 is the primary H2AK119 deubiquitinating enzyme in thymocytes, and loss of BAP1 leads to an arrest at the DN3 stage (9). However, BAP1 was not shown to be required later in T cell development when CD4-cre was used. The mechanism for BAP1-mediated regulation of the DN3 stage has yet to be determined.

## ARGININE METHYLATION

In addition to lysine methylation, histones also undergo arginine methylation, which is associated with transcriptional activation (H4R3me2a, H3R2me2s, H3R17me2a, or H3R26me2a) or repression (H3R2me2a, H3R8me2a, H3R8me2s, or H4R3me2s) (**Figure 2**) (reviewed in 50). Addition of arginine methylation is mediated by the PRMT (protein arginine methyltransferase) family, but thus far no enzyme that only has activity for arginine demethylation has been identified. However, some lysine demethylases can also mediate arginine demethylation (50). PRMTs also mediate arginine methylation of many nonhistone proteins, including proteins involved in mRNA translation, pre-mRNA splicing, and protein stability. Two members of the PRMT family are critical for thymocyte development. CARM1 (also known as PRMT4) is required early in T cell development at the DN1/2 stage for thymocyte survival (135, 136). Total thymocyte cellularity is decreased in the absence of CARM1, which is likely due to lower levels of IL-7R expression on the cell surface (137). PRMT5 is also required for hematopoiesis, and its deletion using Mx1-cre resulted in severe pancytopenia including decreased thymocyte cellularity (138). Conditional deletion of PRMT5 with CD4-cre did not alter conventional T cell development but abrogated iNKT cell development, due to decreased JAK3 and cytokine common gamma ($\gamma$c) chain expression, which was due to altered RNA splicing rather than altered transcription (139).

## SUMMARY AND OUTLOOK

Chromatin-modifying enzymes are critical for appropriate stage-specific gene expression throughout T cell development. There is concurrent expression of multiple family members that serve overlapping or redundant functions, and therefore elucidation of their function in regulating gene expression only becomes apparent when more than one gene is deleted, such as in the case of HDAC1/HDAC2 in regulating genome stability (68, 69), Tcf1/Lef1 in regulating generation of CD4 SP thymocytes (66), UTX/JMJD3 in regulating thymocyte egress (121), or Ring1A/Ring1B in the elimination of B cell potential during T cell commitment and specification (128). Although these chromatin-modifying enzymes are typically thought of as general factors with widespread effects throughout the genome, in many cases the biological effect of the gene deletion observed during T cell development is due to a limited set of dysregulated genes, or just one. For example, the block in positive selection in CD2-icre HDAC3 cKO mice is due to the failure to downregulate ROR$\gamma$t (77), and the block in thymic egress in the absence of UTX/JMJD3 is due to failure to appropriately upregulate S1pr1 (121). In addition, it is also important to consider whether the primary effect of these chromatin-modifying enzymes might be due to modification of nonhistone proteins, such as PRMT5 regulation of JAK3 and $\gamma$c expression through altered RNA splicing (139). In addition, regulation of gene expression by HATs and HDACs may involve acylations other than acetylation.

While the focus here is on enzymes that directly modify histones, the regulation of chromatin structure and accessibility is more complex. Nucleosomes are repositioned by chromatin-remodeling complexes to allow accessibility, and deficiency in key components of many of these complexes blocks T cell development (reviewed in 140–142). Changes in chromatin compartmentalization through topologically associating domains, identified by Hi-C, also regulate chromatin accessibility during T cell development (143). In addition, recent work has demonstrated the critical role of a noncoding RNA, ThymoD, in regulation of chromatin positioning that induces Bcl11b expression at the DN2 stage (144). The Bcl11b locus is repositioned in the nucleus prior to expression, which requires DNA demethylation by TET proteins triggered by expression of ThymoD, which is encoded within the Bcl11b enhancer. The mechanism linking ThymoD to DNA demethylation is not clear, but it appears to depend on local transcription rather than the

primary sequence of ThymoD. DNA demethylation unmasks binding sites for CTCF and cohesin, which mediate chromosomal looping bringing the Bcl11b promoter and enhancer into close proximity. Once the loop is established, the Bcl11b locus is repositioned from a transcriptionally repressive environment near the nuclear envelope into a transcriptionally permissive domain within the nuclear interior, leading to additional changes in chromatin modifications and transcription factor recruitment and subsequent transcriptional activation. Thus, gene regulation is also tied to higher-order chromatin structures and nuclear localization. Understanding of how these factors all work together to regulate gene expression will await further study.

## DISCLOSURE STATEMENT

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