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Tcf1 and Lef1 pack their own HDAC

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Abstract

The transcription factors Tcf1 and Lef1 have intrinsic histone-deacetylase activity that is required for the repression of CD4⁺ T cell-lineage genes in CD8⁺ T cells.

The epigenetic mechanisms that establish and maintain T cell identity are critically important for immune-system function. During the development of conventional $\alpha\beta$ T cells in the thymus, CD4⁺CD8⁺ double-positive thymocytes bifurcate into distinct lineages of CD8⁺ cytotoxic T cells that recognize major histocompatibility complex (MHC) class I, and CD4⁺ helper or regulatory T cells that are specific for MHC class II molecules. Although many transcription factors critical to this process have been characterized, including ThPOK or Runx3 for commitment to the CD4⁺ lineage or CD8⁺ lineage, respectively, less is known about the epigenetic mechanisms involved. DNA methyltransferases are needed to repress expression of the co-receptor CD4 in CD8⁺ T cells, and the histone deacetylases HDAC1 and HDAC2 are needed to repress expression of the co-receptor CD8 in CD4⁺ T cells^{1–3}. In this issue of *Nature Immunology*, Xing *et al.* present the surprising finding that the high-mobility group transcription factors Tcf1 and Lef1 have an intrinsic HDAC domain that acts in cells of the CD8⁺ lineage to repress genes associated with the CD4⁺ lineage and genes encoding products that mediate the effector functions of cytotoxic T cells⁴.

Published studies of mice deficient in Tcf1 and/or Lef1 have identified important functions for these transcription factors in T cell development, including specification of the T cell fate, the proliferation and survival of thymocytes, commitment to the CD4⁺ lineage, and silencing of *Cd4* in CD8⁺ T cells^{5–7}. MHC-class-II-selected thymocytes are redirected from the CD4⁺ lineage to the CD8⁺ lineage in mice in which Tcf1 and Lef1 are conditionally inactivated during thymocyte development by Cre recombinase driven by the *Cd4* regulatory elements (called ‘*Tcf1*^{−/−}*Lef1*^{−/−} mice’ here)⁷. This is due, at least in part, to defective upregulation of ThPOK expression in MHC-class-II-selected thymocytes. In the current work, Xing *et al.* investigate the functions of these transcription factors in the CD8⁺ cytotoxic lineage, in which they note aberrant CD4 expression in *Tcf1*^{−/−}*Lef1*^{−/−} mice⁴.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Gene-expression profiling by RNA-sequencing analysis of mature CD8⁺ thymocytes identifies over 1,000 transcripts expressed differentially in cells from *Tcf7*^{-/-}*Lef1*^{-/-} mice relative to their expression in cells from wild-type mice. Included among the 472 genes upregulated are not only *Cd4* but also those encoding effector molecules of the CD8⁺ lineage, such as perforin, Blimp-1 and Fas ligand, as well as multiple other genes associated with the CD4⁺ lineage, including *Cd40lg*, *FoxP3* and *Rorc*. Expression of these genes persists when MHC class I selection is enforced by crossing of *Tcf7*^{-/-}*Lef1*^{-/-} mice to mice expressing mutant MHC class II. Thus, Tcf1 and Lef1 have important roles in the establishment of both major thymocyte lineages⁴.

To determine how Tcf1 and Lef1 regulate gene expression in CD8⁺ T cells, Xing *et al.* investigate the genome-wide chromatin state of *Tcf7*^{-/-}*Lef1*^{-/-} CD8⁺ T cells by analyzing histone acetylation and methylation via chromatin immunoprecipitation followed by high-throughput sequencing⁴. They find an increase in the acetylation of histone H3 at Lys27 (H3K27Ac) and H3K9Ac at genes aberrantly upregulated in CD8⁺ thymocytes from *Tcf7*^{-/-}*Lef1*^{-/-} mice, at levels approaching those found in wild-type CD4⁺ thymocytes. This is consistent with the association of H3K27Ac and H3K9Ac with active gene expression. Changes in the trimethylation of histone H3 at Lys4 (H3K4Me3), considered an active promoter mark, are more modest, but these also correlate with de-repressed genes of the CD4⁺ lineage. Similar analysis of Tcf1 in wild-type CD8⁺ thymocytes shows that about half of the genes upregulated in the *Tcf7*^{-/-} *efl*^{-/-} CD8⁺ thymocytes are normally bound by Tcf1. Of these direct targets, nearly 80% have greater H3K27Ac in CD8⁺ thymocytes from mutant mice than in those from wild-type mice, which suggests that Tcf1 prevents the acetylation of or mediates the deacetylation of histones at its target loci. These observations prompt the authors to seek a mechanism for how Tcf1 and Lef1 regulate histone acetylation.

Changes in histone acetylation mediated by histone acetyltransferases and HDACs are critical for appropriate lineage-specific gene expression. Acetylated histones are typically associated with open chromatin and active genes, whereas deacetylated histones are associated with compact chromatin and repressed genes. In mammals there are 18 HDACs categorized into four classes on the basis of phylogeny and sequence homology, with class III HDACs (known as the 'sirtuins') considered nonclassical HDACs due to their dependence on the cofactor NAD. These enzymes are recruited to target loci by sequence-specific DNA-binding factors. As previous deletions of HDAC were not reported to result in derepression of CD4 in cells of the CD8⁺ lineage, and there are no changes in the expression of HDACs or histone acetyltransferases in thymocytes of the CD8⁺ lineage upon loss of Tcf1 and Lef1, Xing *et al.* hypothesize that these transcription factors could perhaps directly deacetylate histones at their target loci^{3,8}. Thus, they assay Tcf1 and Lef1 for intrinsic HDAC activity using both proteins translated *in vitro* and recombinant proteins. Both proteins demonstrate HDAC activity, as assessed by a fluorescence-based assay, and Tcf1 is shown to have HDAC activity *in vitro* by immunoblot analysis for acetylated histone H3 and by matrix-assisted laser-desorption-and-ionization mass spectrometry with acetylated H3 peptide⁴. HDAC inhibitors diminish the HDAC activity of Tcf1, albeit at concentrations substantially above the half-maximal inhibitory concentration for conventional HDACs.

The HDAC activity of Tcf1 is subsequently mapped biochemically to a 30-amino-acid region between the β -catenin-binding domain at the amino terminus and the high-mobility group DNA-binding domain at the carboxyl terminus. Structural modeling based on homology demonstrates a strong resemblance of the 30-amino-acid HDAC domain of Tcf1 to the corresponding catalytic region of HDAC8. To investigate an *in vivo* role for the HDAC activity of Tcf1, the authors use a retroviral genetic-complementation approach. They transduce *Tcf7^{-/-}Lef1^{-/-}* bone marrow with a construct encoding Tcf1 either deficient in HDAC activity or with sufficient (wild-type) HDAC activity. They then use these cells to reconstitute irradiated congenic recipient mice. The Tcf1 with mutant HDAC retains its binding to known target DNA but is compromised in its ability to repress genes associated with the CD4⁺ lineage (such as *Foxp3* and *Cd4*) in the transduced CD8⁺ thymocytes. The abundance of H3K27Ac is also elevated at several of the target loci in CD8⁺ cells expressing mutant Tcf1 relative to its abundance in CD8⁺ cells expressing wild-type Tcf1. Although the 'rescue' is incomplete with either wild-type Tcf1 or HDAC-deficient Tcf1, these data demonstrate a requirement for the HDAC domain for full functionality *in vivo*. Interestingly, despite its inability to fully repress the expression of genes associated with the CD4⁺ lineage in CD8⁺ thymocytes, HDAC-deficient Tcf1 largely restores differentiation into the CD4⁺ lineage. This finding suggests that the HDAC activity of Tcf1 might be differentially required in CD4⁺ T cells relative to its requirement in CD8⁺ T cells. It is possible, however, that in each cell type there are intrinsic HDAC-dependent and HDAC-independent functions of Tcf1 (and Lef1) that are not detected by the limited sampling of changes in gene expression and in histone acetylation (Fig. 1). It will therefore be important to determine how the HDAC activity of Tcf1 influences global transcription in thymocytes and mature T cells. Since the deacetylase is required in CD8⁺ T cells for the repression of genes encoding products involved in multiple effector functions, it will also be of interest to determine its role in host protection and inflammatory processes.

The results of Xing *et al.* provide a new twist to the prevalent view that sequence-specific transcription factors regulate histone modification indirectly through recruitment of the relevant enzymes⁴. The newly discovered intrinsic HDAC function of Tcf1 and Lef1 provides an additional layer of regulation that will probably have important implications for the numerous roles ascribed to these proteins not only in the development and effector functions of T cells but also in Wnt signaling and stem-cell biology. The authors propose the exciting prospect that co-activators and co-repressors such as β -catenin and members of the Groucho-TLE family might regulate the intrinsic HDAC activity of Tcf1 and Lef1. It will be important to determine if there are non-histone substrates for deacetylation by Tcf1 *in vivo*. In T cells, Tcf1 has been reported to bind directly to the transcription factor Runx3, which is acetylated in some cancers^{7,9}. Perhaps the HDAC domain of Tcf1 acts directly on Runx3 to influence its role in the establishment of heritable *Cd4* silencing. It will also be interesting to learn whether other transcription factors have intrinsic chromatin-modifying properties and how generalizable these results are to gene regulation. Finally, given the clinical use of HDAC inhibitors for T cell lymphomas and their promising pre-clinical results in a variety of other diseases, there might also be therapeutic opportunities for inhibiting transcription-factor-intrinsic HDAC domains. Characterization of the structures of Tcf1 and Lef1 and their

HDAC domains is now needed to yield more mechanistic insight into the enzymatic properties of these non-conventional transcriptional regulators.

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Figure 1.

Tcf1 and Lef1 regulate gene expression in CD8⁺ thymocytes through intrinsic HDAC activity. The transcription factors Tcf1 and Lef1 contain an HDAC domain that reverses the acetylation of histones and is required for the repression of multiple genes in CD8⁺ T cells (top). In *Tcf1*^{-/-} *Lef1*^{-/-} mice, there is derepression of genes associated with the CD4⁺ lineage and genes encoding effector molecules associated with cytotoxic T cells, in CD8⁺ thymocytes. Complementation of Tcf1- and Lef1-deficient thymocytes with HDAC-deficient Tcf1 fails to fully ‘rescue’ the aberrant gene expression of CD8⁺ thymocytes (middle). There is less dependence on the HDAC domain of Tcf1 for the differentiation of CD4⁺ thymocytes, which raises the possibility of context-dependent requirements for the HDAC domain at lineage-regulating genomic loci. Thus, gene activation at some loci might involve cofactors that inhibit HDAC function (bottom). CBP, histone acetyltransferase.