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Stage-specific epigenetic gene silencing during thymocyte lineage commitment

Ichiro Taniuchi

Address: Laboratory for Transcriptional Regulation, RCAI, RIKEN Centre for Integrative Medical Sciences (IMS-RCAI), 1-7-22, Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045, Japan

Email: taniuchi@rcai.riken.jp

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Abstract

During lineage commitment, precursor cells must establish their signature gene expression programs to endow them with the requisite set of cellular functions. To maintain cellular identity, the gene expression program must be inherited stably by progeny of lineage-committed cells. Epigenetic regulation serves as a central mechanism to maintain such cellular memory. Although a lot of progress has been made in the last decade towards defining the spectrum of epigenetic modifications on histones and DNA, as well as the relevant enzymatic machinery, the mechanisms by which these modifiers are controlled during development remain poorly understood. Gene regulation at the *Cd4* and *Thpok* loci provides ideal models for developmentally regulated gene silencing. A single transcriptional silencer at each locus establishes heritable, irreversible epigenetic silencing only in lineage-committed cells, whereas the same silencer elements establish a reversible repressive state in precursor cells. The dynamic versus permanent silencing of developmentally regulated loci by the stage-specific functions of silencers will be discussed in the context of cell lineage commitment.

Introduction

The inheritance of a gene expression program that persists beyond cell division is essential to maintain lineage identity. In this regard, epigenetic mechanisms play a major role in stable gene repression, referred to as gene silencing [1,2]. Although many of the factors involved in gene silencing have been identified, little is known about the timing of this process during development. Given the necessity of maintaining the plasticity of gene expression in precursors, silencing mechanisms may shift from transient to permanent further along a developmental pathway. Here, I discuss recent advances in our understanding of stage-specific gene silencing at the *Cd4* and *Thpok* loci during thymocyte lineage commitment.

Recent advances

T lymphocytes undergo a highly ordered developmental program in the thymus before they become fully competent "soldiers" of the immune system, defending us against harmful pathogens. Developing thymocytes must also survive two critical selection processes, known as positive selection, during which cells expressing functional T cell antigen receptors (TCR) are selectively maturated, and negative selection, during which "self-reactive" or useless clones are eliminated [3]. After this primary education, T lymphocytes are further taught to have specialized immune functions, a process known as lineage commitment [4]. These lineage-specific properties must be maintained after T lymphocytes egress from the thymus to be positioned at the frontline of our defenses. Accordingly, the gene expression signature for effector functions, which is established during lineage commitment, must be memorized to maintain cell identity [5]. Over the past decade, it has become clear that epigenetic modifications are essential components of cellular memory, driving the inheritance of gene expression states through cell divisions [1,2]. In particular, an impressive variety of histone modifications has been identified, which serves as a flexible code for regulating gene transcription [6], together with the identification of

molecules that contain enzymatic activity for catalyzing these modifications [7]. Another epigenetic modification, DNA methylation at CpG dinucleotides, is also a key component of stable gene repression [8]. New studies are uncovering mechanisms for active DNA de-methylation through a TET-family protein [9,10]. It is also becoming apparent that there is cross-regulation between covalent histone modifications and DNA methylation [11,12]. All of these epigenetic marks can be written and erased by a large arsenal of enzymes, and the resultant modification patterns at a given location are "read" by nuclear factors to modulate gene expression.

A primary mechanism by which epigenetic modifications are translated into gene expression states is through changes in the accessibility of chromatin. Indeed, a densely packed configuration of chromatin, termed heterochromatin, is thought to play an essential role in the stable maintenance of gene repression [13]. Some forms of heterochromatin can be developmentally regulated, permitting more dynamic control of gene expression. A major area of interest is how and when distinct forms of permanent versus dynamic heterochromatin are established, presumably via the action of *cis*-regulatory elements within a given locus.

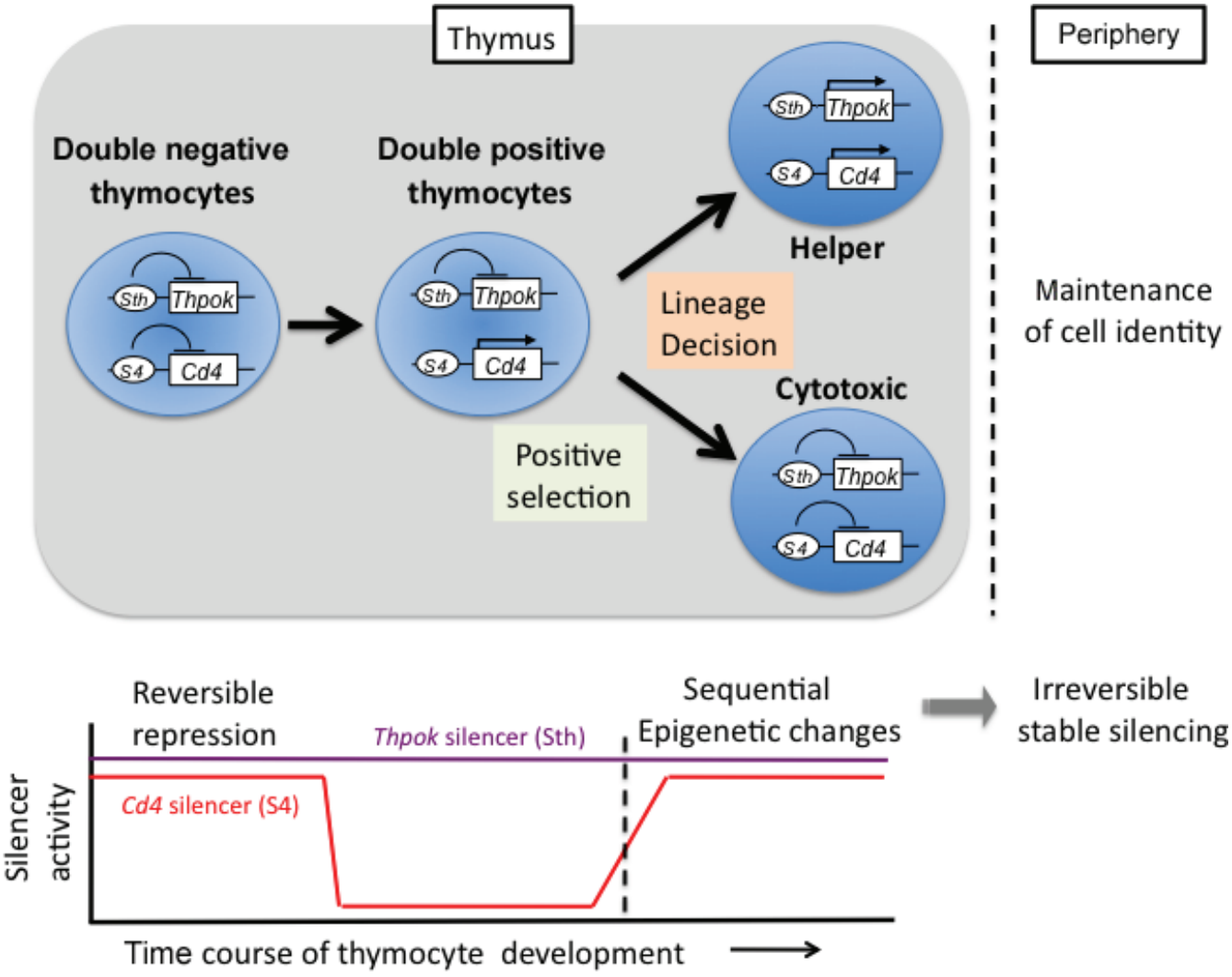
Two genetic loci that have served as excellent models to study these issues encode the CD4 co-receptor and the ThPOK transcription factor, two important lineage-commitment molecules for T lymphocytes [14]. There are four major thymocyte subsets, which can be defined by stage- and lineage-specific expression of CD4 and CD8 co-receptors. Immature thymocyte progenitors that migrate to the thymus express neither CD4 nor CD8, appearing as $CD4^-CD8^-$ double negative thymocytes. A subset of precursor thymocytes express both CD4 and CD8 co-receptors on their surface (and are thereby referred to as $CD4^+CD8^+$ double positive thymocytes) before they differentiate into one of two major subsets, $CD4^+$ $CD8^-$ helper- or $CD4^-CD8^+$cytotoxic-T lymphocytes [15]. Upon exposure to cues for differentiation into the cytotoxic-lineage, the *Cd4* gene is suppressed via the activity of an intronic transcriptional silencer [16,17], generating the $CD4^-CD8^+$ subset. Production of ThPOK is also inhibited in cytotoxic-lineage cells by a transcriptional silencer element in the *Thpok* gene [18,19]. Thus silencers, rather than known enhancer elements, in the *Cd4* and *Thpok* genes share cytotoxic lineage-specific activity to control their lineage-specific expression. Another commonality in these silencers is their usage of Runx transcription factors to exert their function [20,21]. Importantly, using genetic tools that enable us to remove a particular genome region by Cre-mediated site-specific recombination in mice, these *cis*-elements have been shown to be dispensable after precursors are fully committed to the cytotoxic-lineage, reflecting a stable state of repression, although they are required to establish *Cd4* and *Thpok* silencing [21,22]. Thus, these silencers do their specialized job only during a particular developmental time window when lineage-specific repressive state is determined. However, remarkably, both the *Cd4* and *Thpok* silencers have been shown to also function at an earlier stage of thymocyte development [18,22-24]. Repression of *Cd4* in $CD4^-CD8^-$ double negative thymocytes is also regulated by the same silencer element that permanently represses the gene upon commitment to the cytotoxic lineage (see Figure 1).

However, the repressed status of *Cd4* in these early progenitors is reversible, as demonstrated by its activation at the subsequent $CD4^+CD8^+$ stage of development. Similarly, the silencer in the *Thpok* gene turned out to be functional in precursor thymocytes, preventing premature activation of the gene prior to lineage choice. Furthermore, *Thpok* repression in these precursor cells is accompanied by the accumulation of trimethylation at the lysine 27 residue on histone H3, a representative epigenetic mark for a repressive locus [21]. Despite these repressive epigenetic modifications in precursors, the *Thpok* gene is later activated in developing thymocytes toward the helper-lineage. However, similar to the situation I described for *Cd4*, the *Thpok* silencer is required only to maintain repression in precursor cells, but is dispensable after full commitment to the cytotoxic-lineage [21]. Thus, a single silencer element at each of these loci is employed to regulate two distinct modes of gene repression: reversible repression in precursor thymocytes versus irreversible epigenetic gene silencing in fully committed $CD8^+$ cytotoxic T cells. This also suggests that a covalent histone modification (e.g. methylation at lysine 27) that is known to correlate with a repressive state is not necessarily an 'epigenetic' mark for heritable irreversible silencing, at least in the *Thpok* locus. The mechanisms by which a single silencer can induce these two types of repressive chromatin states, depending on the developmental context, remain to be established. Given that lineage commitment can occur only in post-selection thymocytes that have received TCR-mediated positive selection signals, a plausible scenario is that TCR-based signals may induce new epigenetic pathways that target chromatin associated with *Cd4* and *Thpok* for additional modifications that establish permanent repression.

Summary

Although the molecular mechanisms that underlie stage-specific epigenetic silencing remain elusive, the physiological relevance of this process is clear. Given the essential

Figure 1. Distinct modes of gene repression by the same silencer at distinct stages



The expression state of the *Cd4* and *Thpok* genes at distinct stages of thymocyte development is illustrated at the top. Relative activities of the *Cd4* silencer (S4, red line) and *Thpok* silencer (Sth, purple line) during thymocyte differentiation towards the cytotoxic lineage are illustrated at the bottom. Whereas both *Cd4* and *Thpok* genes are repressed in immature double negative thymocytes, the *Cd4*, but not *Thpok*, gene is expressed in double positive thymocyte precursors. After passing a selection process known as "positive selection", thymocytes choose either a helper or a cytotoxic fate. After this lineage decision, selected cellular identity is maintained, which requires stable inheritance of gene expression status by progeny cells. In order to achieve this, both the *Cd4* and *Thpok* genes receive sequential epigenetic changes through the silencer, eventually resulting in the establishment of gene silencing that can be inherited in the absence of the silencer. Thus, the same silencer can induce reversible gene repression in precursors and irreversible gene silencing in fully committed $CD8^+$ cytotoxic T cells.

requirement for ThPOK expression in helper T cell commitment [25], a premature, irreversible repression of *Thpok* in precursor thymocytes would preclude the generation of helper-lineage cells. Instead, the repressive state of *Thpok* in precursors must retain plasticity for subsequent gene activation. Thus, a reversible repression at the double positive stages provides flexibility that allows the generation of CD4 and CD8 lineages. Once these precursors are exposed to positive selection signals, the plasticity must be transformed to a permanent state of repression in $CD8^+$ cytotoxic T cells or the silencer element is disarmed to allow *Thpok* gene activation in $CD4^+$ helper T cells. However, there is evidence that epigenetic sealing of the *Thpok* locus may occur independently from commitment to the cytotoxic T-lineage. Specifically, potentiation of silencer activity at the *Thpok* locus by increasing the copy number of the silencer element from one to three mediates heritable *Thpok* silencing even in helper-lineage T cells [21]. Future studies to address this and other mechanisms of silencing at the *Cd4* and *Thpok*

loci will provide important insights for solving the enigma of how a single silencer element controls reversible and irreversible modes of gene repression at appropriate stages of development.

Abbreviations

TCR, T cell antigen receptor.

Disclosures

The author declares that he has no disclosures.

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References

1. Bonasio R, Tu S, Reinberg D: **Molecular signals of epigenetic states.** *Science* 2010, **330:**612-616.
2. Probst AV, Dunleavy E, Almouzni G: **Epigenetic inheritance during the cell cycle.** *Nat Rev Mol Cell Biol* 2009, **10:**192-206.
3. Germain RN: **T-cell development and the CD4-CD8 lineage decision.** *Nat Rev Immunol* 2002, **2:**309-322.
4. Singer A, Adoro S, Park JH: **Lineage fate and intense debate: myths, models and mechanisms of CD4- versus CD8-lineage choice.** *Nat Rev Immunol* 2008, **8:**788-801.
F1000Prime RECOMMENDED
5. Rothenberg EV, Dionne CJ: **Lineage plasticity and commitment in T-cell development.** *Immunol Rev* 2002, **187:**96-115.
F1000Prime RECOMMENDED
6. Jenuwein T, Allis CD: **Translating the histone code.** *Science* 2001, **293:**1074-1080.
F1000Prime RECOMMENDED
7. Black JC, Van Rechem C, Whetstine JR: **Histone lysine methylation dynamics: establishment, regulation, and biological impact.** *Mol Cell* 2012, **48:**491-507.
F1000Prime RECOMMENDED
8. Jones PA: **Functions of DNA methylation: islands, start sites, gene bodies and beyond.** *Nat Rev Genet* 2012, **13:**484-492.
9. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A: **Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1.** *Science* 2009, **324:**930-935.
F1000Prime RECOMMENDED
10. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y: **Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification.** *Nature* 2010, **466:**1129-1133.
F1000Prime RECOMMENDED
11. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A: **Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex.** *Nature* 1998, **393:**386-389.
F1000Prime RECOMMENDED
12. Ng HH, Zhang Y, Hendrich B, Johnson CA, Turner BM, Erdjument-Bromage H, Tempst P, Reinberg D, Bird A: **MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex.** *Nat Genet* 1999, **23:**58-61.
F1000Prime RECOMMENDED
13. Henikoff S: **Heterochromatin function in complex genomes.** *Biochim Biophys Acta* 2000, **1470:**O1-8.
F1000Prime RECOMMENDED
14. Taniuchi I, Ellmeier W: **Transcriptional and epigenetic regulation of CD4/CD8 lineage choice.** *Adv Immunol* 2011, **110:**71-110.
15. Singer A, Bosselut R: **CD4/CD8 coreceptors in thymocyte development, selection, and lineage commitment: analysis of the CD4/CD8 lineage decision.** *Adv Immunol* 2004, **83:**91-131.
16. Sawada S, Scarborough JD, Killeen N, Littman DR: **A lineage-specific transcriptional silencer regulates CD4 gene expression during T lymphocyte development.** *Cell* 1994, **77:**917-929.
F1000Prime RECOMMENDED
17. Siu G, Wurster AL, Duncan DD, Soliman TM, Hedrick SM: **A transcriptional silencer controls the developmental expression of the CD4 gene.** *Embo J* 1994, **13:**3570-3579.
F1000Prime RECOMMENDED
18. Setoguchi R, Tachibana M, Naoe Y, Muroi S, Akiyama K, Tezuka C, Okuda T, Taniuchi I: **Repression of the transcription factor Th-POK by Runx complexes in cytotoxic T cell development.** *Science* 2008, **319:**822-825.
19. He X, Park K, Wang H, He X, Zhang Y, Hua X, Li Y, Kappes DJ: **CD4-CD8 lineage commitment is regulated by a silencer element at the ThPOK transcription-factor locus.** *Immunity* 2008, **28:**346-358.
F1000Prime RECOMMENDED
20. Taniuchi I, Osato M, Egawa T, Sunshine MJ, Bae SC, Komori T, Ito Y, Littman DR: **Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development.** *Cell* 2002, **111:**621-633.
F1000Prime RECOMMENDED
21. Tanaka H, Naito T, Muroi S, Seo W, Chihara R, Miyamoto C, Kominami R, Taniuchi I: **Epigenetic Thpok silencing limits the time window to choose CD4 helper-lineage fate in the thymus.** *Embo J* 2013.
22. Zou YR, Sunshine MJ, Taniuchi I, Hatam F, Killeen N, Littman DR: **Epigenetic silencing of CD4 in T cells committed to the cytotoxic lineage.** *Nat Genet* 2001, **29:**332-336.
23. Taniuchi I, Sunshine MJ, Festenstein R, Littman DR: **Evidence for distinct CD4 silencer functions at different stages of thymocyte differentiation.** *Mol Cell* 2002, **10:**1083-1096.
24. Leung RK, Thomson K, Gallimore A, Jones E, Van den Broek M, Sierro S, Alsheikhly AR, McMichael A, Rahemtulla A: **Deletion of the CD4 silencer element supports a stochastic mechanism of thymocyte lineage commitment.** *Nat Immunol* 2001, **2:**1167-1173.
F1000Prime RECOMMENDED
25. He X, Dave VP, Zhang Y, Hua X, Nicolas E, Xu W, Roe BA, Kappes DJ: **The zinc finger transcription factor Th-POK regulates CD4 versus CD8 T-cell lineage commitment.** *Nature* 2005, **433:**826-833.
F1000Prime RECOMMENDED