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Interplay of transcription factors in T-cell differentiation and function: the role of Runx

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Summary

Over the past years, increasing numbers of distinct subsets have been discovered and identified for a T lymphocytes' entity. Differentiation and function of each T cell subset are controlled by a specific master transcription factor. Importantly, Runt-related transcription factors, particularly Runx1 and Runx3, interplay with these master regulators in various aspects of T cells' immunity. In this review article, we first explain roles of Th-Pok and Runx3 in differentiation of CD4 versus CD8 single positive cells, and later focus on cross-regulation of Th-Pok and Runx3 and their relationship with other factors such as TCR strength. Next, we provide evidences for the direct interplay of Runx1/3 with T-bet and GATA3 during Th1 versus Th2 commitment to activate or silence transcription of signature cytokine genes, *IFNγ* and *IL4*. Lastly, we explain feed-forward relationship between Runx1 and Foxp3 and discuss roles of Runx1 in regulatory T cells' suppressive activity. This review highlights an essential importance of Runx molecules in controlling various T cell subsets' differentiation and functions through molecular interplay with the master transcription factors in terms of protein-protein interaction as well as regulation of gene expression.

Keywords: cell differentiation; gene expression; gene targeting; T lymphocytes; transcription factor

Introduction

In this brief review article, we focus on the role of Runt-related transcription factors, Runx, in the differentiation and function of T lymphocytes. Runx has emerged as one of the most important regulatory factors in T-cell immunity. The Runx family is composed of three members, Runx1, Runx2 and Runx3, each of which forms a functional complex with a core binding factor β (Cbfβ) partner protein. Runx1 and Runx3 are known to be involved in T-cell immunity. T lymphocytes differentiate into subsets with distinct functions. In some T-cell subsets, Runx1 and Runx3 are equivalently expressed and exhibit redundant activities. However, in other T-cell subsets, Runx1 and Runx3 exert distinct functions. These differences depend mainly on the unique expression patterns of these proteins in a particular T-cell subset, but qualitative differences in Runx1 and Runx3 may also contribute to their differential activity in subsets of T cells. In a previously published review article, we described these common and distinct features of Runx factors that are relevant to each step of T-cell differentiation.[1] In the present review, we emphasize the roles of Runx factors in three biologically and clinically important aspects of T-cell differentiation/function. These include lineage selection of helper versus killer cells, and T helper differentiation into various T helper cell and regulatory T-cell subsets.

Roles of Runx3 and Th-POK in killer versus helper lineage commitment

$CD4^+$ $CD8^+$ double-positive (DP) thymocytes ultimately differentiate into two lineages, $CD4^+$ $CD8^-$ single-positive (CD4SP) helper T cells and $CD4^-$ $CD8^+$ single-positive (CD8SP) killer T cells. Extensive studies of the molecular mechanisms and gene regulation involved in this lineage determination have led to the discovery of T helper-inducing POZ-Krüppel-like factor (Th-POK) as a key transcription factor in CD4SP commitment,[2,3] and of Runx3 in CD8SP commitment.[4–6] Furthermore, recent works have revealed that the interplay between Th-POK and Runx3 is pivotal in this thymocyte lineage determination process (Fig. 1).

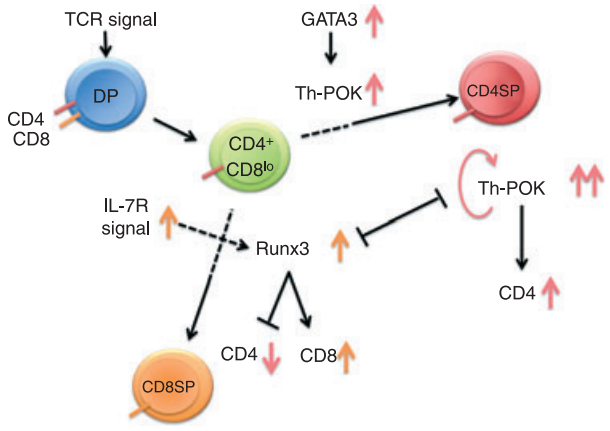

Figure 1. Roles of Runx3 and T helper-inducing POZ-Krüppel-like factor (Th-POK) in the differentiation of CD4 single-positive (SP) and CD8SP thymocytes. Double-positive (DP) cells first move to the $CD4^+$ $CD8^{lo}$ stage, after which they differentiate into the CD4SP lineage if they receive a stronger/longer T-cell receptor (TCR) signal. Induction of *Th-POK* via GATA3 and maintenance of *Th-POK* through a positive auto-regulation mechanism are important in this step. If cells receive a weaker/shorter TCR signal, they differentiate into the CD8SP lineage. The interleukin-7 receptor (IL-7R) signal is somehow linked to the TCR signal for the induction of *Runx3* expression. Runx3 then suppresses *CD4* expression and simultaneously up-regulates *CD8* expression. Runx3 and Th-POK mutually repress each other's expression.

Th-POK in CD4SP differentiation

In 1998, a naturally occurring mutant mouse strain termed helper deficient (HD) was reported, and was so called because these mice lack CD4SP helper T cells.[7] In these mice, MHC class II-restricted thymocytes that would ordinarily become CD4SP are re-directed into the CD8SP lineage. The *HD* mutation does not simply impair the differentiation of CD4SP cells but perturbs the choice between CD4SP and CD8SP lineages. In 2005, it was discovered that the HD phenotype could be attributed to a point mutation in the *Zbtb7* gene, which encodes a zinc finger-containing transcription factor.[2,3] This gene, also known as *cKrox* or *Zfp67*, is now most often referred to as *Th-POK*. Expression of *Th-POK* is limited to the CD4SP lineage, but is not detected in double negative (DN), DP and CD8SP cells. Over-expression of *Th-POK* can force class-I restricted cells that are destined to become CD8SP to re-differentiate into the CD4SP lineage. These observations, together with the phenotype of HD mice, strongly indicate that Th-POK is a master regulator of CD4SP differentiation.

Runx3 in CD8SP differentiation

The Runx3 transcription factor, on the other hand, is a master regulator of CD8SP thymocyte differentiation. *Runx3* deficiency causes a reduction in CD8SP thymocytes; however, this decrease is not as drastic as the reduction of CD4SP seen in *Th-POK*-deficient thymuses. In wild-type thymuses, expression of Runx3 protein is confined to the CD8SP subset, but Runx1 can be detected in all DN, DP, CD4SP and CD8SP cells.[5] Therefore, lack of Runx3 in CD8SP cells probably causes a compensatory increase in Runx1 expression, thereby preventing a severe reduction in CD8SP in *Runx3*-deficient thymuses. When DP cells move to the CD8SP lineage, Runx3 suppresses *CD4* expression by binding to the silencer region of the gene.[4] It must be noted that along the DP to CD8SP pathway, expression of CD8 is first down-regulated (the $CD4^+$ $CD8^{lo}$ stage), but is subsequently re-activated accompanying CD4 repression (the $CD4^-$ $CD8^+$ stage). Runx3 mediates the re-activation of *CD8* expression by binding to the enhancer region of the *E8I* gene.[5] Hence, Runx3 regulates *CD4* and *CD8* expression negatively and positively, respectively, and provides a basis for mutually exclusive expression of CD4 and CD8 in the CD8SP subset.

Cross-regulation of Runx3 and Th-POK

In addition to regulating *CD4* and *CD8* expression, Runx3 can also indirectly regulate these genes by controlling *Th-POK* expression. Runx3 suppresses *Th-POK* expression by binding to its silencer region, which maps to a 3.1 kb upstream region of exon Ia (called RBS-1)[8] containing a Runx consensus site, or to a distal responsive element.[9] In cases of *Runx3* deficiency, *Th-POK* expression is therefore de-repressed, which contributes to the re-direction of class I-restricted or CD8SP-oriented cells into the CD4SP lineage. Conversely, Th-POK can suppress *Runx3* expression by binding to the *Runx3* distal promoter.[10,11] Therefore, both Th-POK and Runx3 are negative regulators of each other's expression.[10]

This poses the question about what would happen to T-cell differentiation if neither Th-POK nor Runx3 were present? Unexpectedly, a substantial number of CD4SP and CD8SP cells are detected in both *Th-POK*- and *Runx3*-targeted thymuses.[10] Therefore, Th-POK is probably not the sole master regulating the choice between the CD4 and CD8 lineage. In addition, the differentiation into the CD8SP lineage is probably not a default step that occurs automatically without the guidance of transcription factors. Runx3 appears to have functional significance other than its role in silencing *Th-POK* expression.

Various lines of evidence have indicated that the strength and duration of T-cell receptor (TCR) signals determines the lineage selection of DP thymocytes. Namely, a stronger and longer signal induces CD4SP differentiation, whereas a weaker and shorter signal results in CD8SP differentiation.[12] Therefore, it is reasonable to link the stronger/longer TCR signal to *Th-POK* expression

and the weaker/shorter signal to *Runx3* expression. Expression of Th-POK protein inside cells is primarily determined at the transcriptional level. *Th-POK* transcription is initially induced by GATA3, whereas maintenance of *Th-POK* transcription relies on the Th-POK protein itself (positive auto-regulation).[11,13] Therefore, the TCR signal together with GATA3 may induce *Th-POK* expression, while the strength/length of the TCR signal may stabilize *Th-POK* expression. In contrast, interleukin-7 receptor (IL-7R) and its downstream signalling molecule STAT5 are reported to induce *Runx3* expression.[14] It is not known at present how IL-7R signalling is linked to the weaker/shorter TCR signal.

In essence, Runx3 and Th-POK not only function antagonistically in the regulation of CD4/CD8 expression but also exert mutually suppressive activity on their own expression (Fig. 1). However, the activity attributed to Runx3 and Th-POK is necessary but not sufficient for lineage selection. For example, Runx3-binding to RBS-1 does not guarantee suppression of *Th-POK* expression.[8] This suggests that some unknown factor other than Runx3/Th-POK might be involved in CD4SP/CD8SP lineage selection.

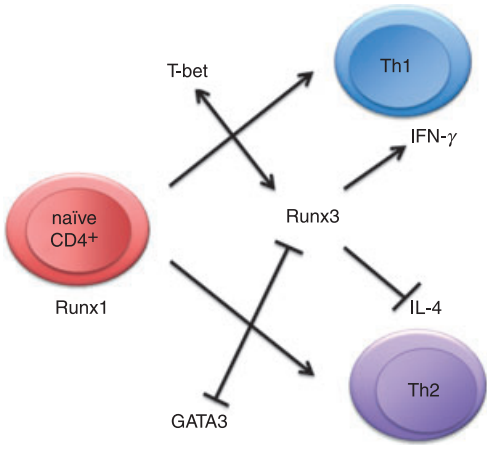

Figure 2. Roles of Runx factors in T helper differentiation. In naive $CD4^+$ cells, Runx1 is detected, but its expression is down-regulated following T-cell receptor stimulation. Artificial abrogation of Runx1 skews the cells to a T helper type 2 (Th2) phenotype. However, Runx3 is induced in the Th1 differentiation pathway, and the Runx3–T-bet complex functions to enhance *IFNγ* and repress *IL4* expression. Runx3 also interacts with and attenuates the activity of GATA3.

Roles of Runx, T-bet and GATA3 in T helper type 1 versus type 2 commitment

When encountering foreign antigens, peripheral $CD4^+$ T cells initiate differentiation towards T helper type 1 (Th1), Th2, or other helper lineages, depending on the types of antigens encountered. The Th1 and Th2 phenotypes are characterized by the secretion of the representative cytokines interferon-γ (IFN-γ) and IL-4, which are controlled by their master regulators, the T-bet and GATA3 transcription factors, respectively. Recent advances have identified Runx factors as important regulators of T helper differentiation (Fig. 2).

Expression profiles of Runx proteins during T helper differentiation

Runx1, but not Runx3, is found in naive $CD4^+$ T cells whose TCR stimulation immediately down-regulates Runx1 protein expression.[15] Interestingly, when cells are cultivated under conditions that skew their T helper differentiation, Runx1 is re-activated or the expression of Runx3 is newly induced. Hence, Th1-committed cells express only Runx3, whereas Th2 cells express both Runx1 and Runx3.[15–18]

Runx1 represses *GATA3* and *IL4* expression in naive $CD4^+$ cells

Is modulation of the expression pattern of Runx an obligatory step for T helper differentiation? A potential answer to this question has been furnished by a report about Runt-transgenic cells.[15] The Runt domain is the DNA-binding domain of Runx, and functions in a dominant negative fashion against the intact Runx protein. Upon TCR activation, the differentiation of Runt-transgenic $CD4^+$ cells is more skewed towards the Th2 phenotype than that of non-transgenic cells. This effect has been attributed to the up-regulation of GATA3 expression. However, transduction of Runx1 into naive wild-type cells attenuates Th2 skewing, and is accompanied by cessation of GATA3 expression. Runx1 also affects Th differentiation by binding to a DNaseI hypersensitive site IV in the *IL4* silencer, thereby repressing *IL4* expression.[16] Therefore, a drop in the level of Runx1 expression after TCR stimulation might be a prerequisite for naive $CD4^+$ cells to commence Th2 differentiation.

Runx3 and T-bet co-operatively augment Th1 differentiation

We next focus on the induction of Runx3 expression during T helper differentiation. When $CD4^+$ cells are cultured under Th1-skewing conditions, T-bet is initially induced, and Runx3 is subsequently up-regulated in a T-bet-dependent manner.[17] T-bet and Runx3 proteins have been shown to physically associate to form the T-bet/Runx3 complex which then binds to the *IL4* silencer (hypersensitive site IV) to repress *IL4* expression, and to the *IFNγ* promoter to enhance *IFNγ* expression. It is notable that Runx3 is up-regulated at a rather late stage of TCR activation under Th1-skewing conditions. A

positive, feed-forward interplay of Runx3 and T-bet is therefore likely to be involved in enforcement of commitment to the Th1 lineage, but not in the determination of the lineage itself. Runx3 protein also appears to contribute to the strength of the Th1 phenotype by physically interacting with and attenuating the activity of GATA3 protein.[18] In other words, GATA3 can block Runx3-mediated *IFNγ* expression by interacting with Runx3.[19] In contrast to Th1 cells, the significance of Runx1 and Runx3 expression in Th2-skewed cells is not clear. One possibility is that Runx functions to down-modulate excessive Th2 reactivity.

Disturbance of the Th1/Th2 balance in *Runx*-modulated mice

Collectively, both Runx1 and Runx3 attenuate Th2 and favour Th1 phenotypes so *Runx*-abrogated mice with the *Cbfb*$^{f/f}$*;CD4-Cre-tg* mutation are prone to Th2-type diseases such as bronchial asthma,[16] in which inflammation along the airway and elevated serum levels of IgA, IgG1 and IgE (Th2-associated immunoglobulin subclasses) are observed. In contrast, when *Runx3* is transduced into mice as a transgene, expression of Th1-type cytokines (IFN-γ, tumour necrosis factor-α, IL-12 and IL-18) is uniformly increased even in unstimulated CD4$^+$ cells.[18] Antigen injection of *Runx3-tg* mice results in higher serum levels of IgG2a and IgG2b (Th1-associated immunoglobulin subclasses).

Runx1 and Th17

Th17 is another distinct T helper subset into which CD4$^+$ cells differentiate, and RORγt is a master regulator of this Th17 lineage.[20] As for the involvement of Runx1 in Th17 differentiation, retroviral transduction of *Runx1* has been shown to induce the Th17 phenotype, whereas *Runx1* siRNA antagonizes Th17 differentiation.[21] Runx1 appears to favour the Th17 phenotype by forming a complex with RORγt, and up-regulates *IL17* expression by binding to its enhancer and promoter. Runx1 promotes *RORγt* expression as well. It remains to be seen if these observed effects of Runx1 on Th17 differentiation also hold *in vivo*, using *Runx1*-engineered mice.

Differentiation and function of regulatory T cells

Excessive immune activity against self or foreign antigens is suppressed by a subset of regulatory CD4$^+$ T (Treg) cells whose activity prevents autoimmune disease by increasing tolerance towards the antigen. Treg cells, which are characterized by high expression of the Foxp3 transcription factor, arise naturally as Treg cells (nTreg) in the thymus, or as induced Treg cells (iTreg) in peripheral lymphoid tissues.[22–24] A sustained high level of Foxp3 expression is vital for expression of various Treg-cell-associated genes as well as for differentiation and maintenance of Treg cells.[25] Mutations in the *Foxp3* gene lead to a catastrophic autoimmune disease termed IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome in humans or to the scurfy phenotype in mice.[26,27]

Pathologies observed in mice with *Runx1*-deficient Treg cells

The function of the Runx1/Cbfβ complex in Treg cells has been intensively investigated. An *in vitro* study demonstrated that the physical interaction of Runx1 with FoxP3 is necessary for Treg cells to exert their suppressive activity on immunological reactions.[28] Subsequently, two *in vivo* studies confirmed that the Runx1/Cbfβ complex is indispensable for Treg-cell function. In one report,[29] *Runx1*$^{F/F}$*:FIC* and *Cbfβ*$^{F/F}$*:FIC* mice were generated in which *Runx1* or *Cbfβ* was targeted specifically in Treg cells. These mice develop gastritis with elevated serum IgE, autoantibodies, splenomegaly and lymphadenopathy. In another report,[30] *Cbfβ*$^{fl/fl}$*:Foxp3*$^{YFP\text{-}cre}$ mice harbouring *Cbfβ*-deleted Treg cells were generated. These mice showed lymphohistocyte and plasmacyte infiltration into multiple organs, and developed inflammatory diseases, such as pneumonitis and arteritis in the lungs. It is noteworthy that the pathologies observed in Treg-specific *Runx1*- and *Cbfβ*-deficient mice are similar to, but relatively milder than those seen in *FoxP3*-deficient mice.[22]

Runx1 is essential for the suppressive action of Treg cells

Is the occurrence of autoimmune disease in the aforementioned Treg-cell-specific, *Runx1/Cbfβ*-deleted mice attributable to compromised, suppressive Treg-cell activity? Adoptive transfer of *Cbfβ*-deficient Treg cells into severe combined immunodeficiency disorder (SCID) mice failed to prevent the development of CD4$^+$ CD25$^-$ CD45RB^{hi} cell-induced colitis.[29] When cultured *in vitro*, *Cbfβ*-deficient Treg cells failed to suppress the proliferation of responder CD4$^+$ T cells.[29–31] Furthermore, knockdown of *Runx1* with small interfering (si) RNA impaired the *in vitro* suppressive activity of human primary or induced Treg cells.[28,32] These observations indicate that the abrogation of Runx1/Cbfβ impairs Treg-cell suppressive activity. Hence, in mice with *Runx1*- or *Cbfβ*-deleted Treg cells, the number of active CD4$^+$ Foxp3$^-$ conventional T cells is increased.[29,30] Such activated T cells tend to secrete pro-inflammatory cytokines such as IFN-γ and IL-4, which presumably drive vicious cycles of inflammation, eventually causing destructive hyper-reactivity.

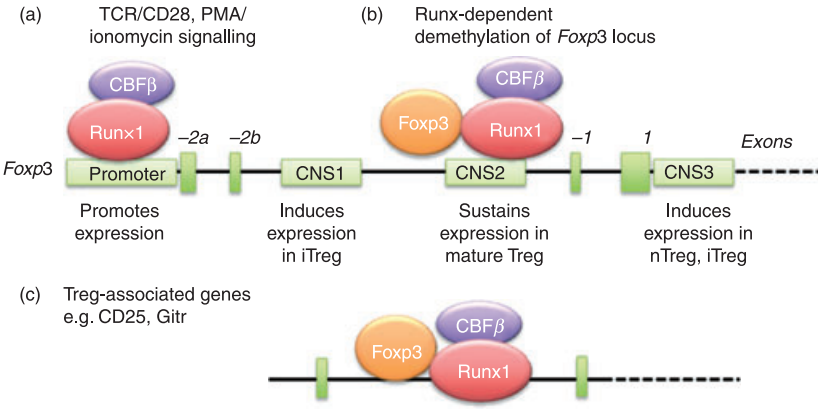

Figure 3. The Runx1/Cbf*β* complex regulates *Foxp3* expression in regulatory T (Treg) cells through a complicated feed-forward mechanisms. *Foxp3* regulatory elements are schematically illustrated, including a promoter and three conserved non-coding sequences (CNS). CNS1 is important for induction of *Foxp3* expression in induced Treg cells, CNS2 maintains sustained *Foxp3* expression in mature Treg cells, and CNS3 induces *Foxp3* expression in both natural Treg and induced Treg cells. Multiple Runx consensus sites have been identified in the promoter and CNS2 regions. (a) Runx1/Cbf*β* binds to a *Foxp3* promoter in response to T-cell receptor signalling. Other transcription factors such as NFAT and AP1 (not shown) are also involved in driving *Foxp3* expression. (b) Runx1/Cbf*β* participates in demethylation of the *Foxp3* locus. Upon demethylation, Foxp3 protein forms a complex with Runx1/Cbf*β* that then binds to the demethylated CNS2 region in mature Treg cells and maintains sustained *Foxp3* expression. (c) Foxp3 together with Runx1/Cbf*β* binds to and regulates the expression of Treg-associated target genes, such as *CD25* and *GITR*. Dark green boxes: exons, light green boxes: regulatory DNA elements.

Regulation of *Foxp3* expression by Runx1

How does the Runx1/Cbf*β* complex regulate Treg cells? Accumulated evidence supports a feed-forward relationship between Runx1/Cbf*β* and Foxp3 (Fig. 3). Namely, Runx1/Cbf*β* induces *Foxp3* gene expression, and subsequently, Runx1/Cbf*β* interacts with Foxp3 protein to regulate expression of *Foxp3* itself as well as other Treg-cell-associated genes.[32] We will first discuss the regulation of *Foxp3* by Runx1/Cbf*β*.

In peripheral iTreg cells derived from $Cbf\beta^{fl/fl}$:$Foxp3^{YFP\text{-}cre}$ or $Cbf\beta^{F/F}$:*FIC* mice, expression of *Foxp3* is substantially reduced.[29,30] Similarly, treatment of human $CD4^+$ $CD25^{hi}$ T cells with *Runx1*-siRNA significantly attenuated expression of *Foxp3*, and this effect was even stronger when cells were treated with both *Runx1*- and *Runx3*-siRNA.[29,31] However, retroviral transduction of *Runx3* into naive $CD4^+$ T cells can elevate Foxp3 protein levels.[32] Above, we discussed the indispensable role of Runx/Cbf*β* in Treg-cell suppressive activity. Interestingly, transduction of *Foxp3* into *Cbfβ*-deficient Treg cells restores their suppressive activity[30], suggesting that Runx/Cbf*β* contributes to Treg-cell function indirectly through the regulation of *Foxp3* gene expression.

Foxp3 gene transcription is controlled by a promoter region (Fig. 3a) and by three conserved non-coding sequences (CNS) in introns (Fig. 3b).[33] The *Foxp3* promoter contains three putative Runx sites at 333, 287 and 53 bp upstream of the transcriptional start site. Runx1 binds to these sites as demonstrated by promoter enzyme immunoassay, promoter tiling and chromatin immunoprecipitation assays.[29–32] In a luciferase reporter assay using PMA/ionomycin-stimulated human $CD4^+$ T cells, transduction of Runx1 greatly enhanced *Foxp3* promoter activity, whereas mutation of the Runx consensus sites abrogated that activity.[29,31]

Runx1/Cbf*β* also binds to the CNS2 element,[29,30] which contains CpG islands and is a differentially methylated region. In footprint analysis using Treg cells, consensus Runx sites in *Foxp3* were accessible to DNaseI, suggesting an open structure for CNS2.[32] The CNS2-bound Runx1 appears to regulate *Foxp3* expression by modifying its chromatin structure. When H3K4me3 or H3K4me9 modifications of histone H3 were evaluated, CNS2 was more methylated in *Cbfβ*-deficient Treg cells than in wild-type Treg cells.[30] In other words, CNS2 is demethylated in a Cbf*β*-dependent manner in wild-type Treg cells. This demethylation appears to allow Foxp3 to bind to CNS2, which then probably contributes to the maintenance of Foxp3 expression.[32,33] As expected, Foxp3 binding to CNS2 is impaired in *Cbfβ*-deficient cells.[33] The above observations collectively suggest a crucial role for Runx1/Cbf*β* in the maintenance of demethylation at the *Foxp3* locus, and by inference, in increased Foxp3 expression in Treg cells.

Runx1-Foxp3 proteins interact to regulate Treg cells

Runx1 and Foxp3 proteins also exert their activities by forming a complex together (Fig. 3c). The Runx1–Foxp3 interaction was demonstrated by co-immunoprecipitation

Table 1. Summary of the roles of Runx in the differentiation and function of T lymphocytes

T-cell subsets	Roles of Runx	Refs mentioned in the text	Refs not mentioned in the text
Reviews	On Runx and T, in general	1	
Reviews	On $CD4^+$ versus $CD8^+$		35–38
DN thymocytes	Runx1 is necessary at multiple steps in the differentiation of DN thymocytes. Runx3 is not a main factor		39–44
DP thymocytes	Runx1 influences apoptotic sensitivity of DP thymocytes		45
$CD4^+$ cells	Runx1 is important for the differentiation of $CD4^+$ thymocytes and homeostasis of peripheral $CD4^+$ cells		42,46
$CD8^+$ cells	Both Runx3 and Runx1 are necessary for the differentiation of $CD8^+$ cells	4–6,14	40,42,47–50
$CD4^+$ versus $CD8^+$ selection, Runx and ThPOK	A transcription factor network is involved in the selection of either the $CD4^+$ or $CD8^+$ lineage	8–11,13	51
Th1/Th2	Runx1 and Runx3 function in favour of Th1 differentiation and antagonize Th2 differentiation	15–19	
Th17	Runx1 and RORγt interact with each other	21	
Treg	Foxp3 segregates the Treg lineage from the conventional $CD4^+$ lineage by interacting with Runx1	28–33	
$\gamma\delta$T and natural killer T	Runx3 is necessary for the emergence of skin-residing $\gamma\delta$T cells. Runx1 is necessary for the development of natural killer T cells in thymus		52,53
Natural killer and dendritic cells	Runx3 is important for the full maturation of the natural killer cell lineage. Runx3 negatively regulates dendritic cell maturation		54–56
Domain analyses of Runx	Intra-Runx domains necessary for CD4 repression		57–60
Runx expression	Runx expression in T cells is regulated transcriptionally and post-transcriptionally		61–63

DN, double-negative; DP, double-positive; Th1, T helper type 1; Th-POK, T helper-inducing POZ-Krüppel-like factor; Treg, regulatory T.

experiments and by *in vitro* glutathione S-transferase pulldown assays.[28] Studies using deletion mutants demonstrated that the regions responsible for the interaction were located between amino acids 362–402 (C-terminal) of Runx1 and 278–336 (between the forkhead domain and the leucine zipper motif) of Foxp3. Transduction of full-length Foxp3 (but not Foxp3 lacking a Runx1 interaction domain) can induce expression of Treg-associated cell surface molecules [CD25, cytotoxic T-lymphocyte antigen 4 (CTLA-4) and glucocorticoid induced tumour necrosis factor receptor (GITR)]. This is because both Runx1 and Foxp3 bind to intron 1 of *CD25* and *GITR*, and work co-operatively in gene induction.[28,34] The above data collectively suggest that the Runx1–Foxp3 complex induces expression of Treg-cell-associated genes (and exerts Treg-cell-suppressive activity). It is noteworthy that, in Th17 cells, Foxp3 forms a complex with Runx1 and RORγt to suppress IL-17 production.[21]

In short, the indispensability of Runx1/Cbfβ for Treg-cell differentiation and stability has been gradually unveiled. Runx1/Cbfβ exerts its effects through a unique feed-forward relationship with a Treg-cell signature transcription factor, Foxp3.

Involvement of Runx transcription factors in other aspects of T-cell differentiation/function

Finally, we summarize the roles of Runx factors in T-cell biology that have been published so far with references that are classified according to the steps of T-cell differentiation/function involved (Table 1). The involvement of Runx in non-canonical $\gamma\delta$T, natural killer T, natural killer and dendritic cell subsets is also summarized. This information provides an up-to-date snapshot of the interplay between T-cell differentiation and function and Runx that should be useful for researchers working in and around this field.

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Disclosures

None.

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