Switch hitter: Bcl11b in T cells and ILC2s

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INSIGHTS

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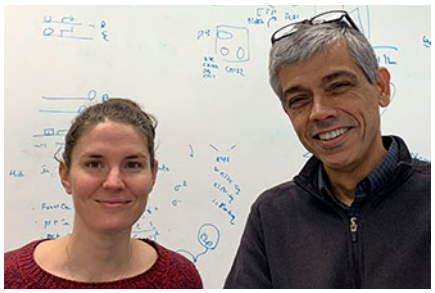
In this issue of *JEM*, Hosokawa et al. (https://doi.org/10.1084/jem.20190972) establish that transcription factor Bcl11b regulates almost completely distinct sets of genes in T cell precursors and ILC2s. To understand how this occurs, they identify multiple levels of functional regulation for Bcl11b that are used differently by T cell precursors and ILC2s.

Innate lymphoid cells (ILCs) were recently identified as the innate counterpart of adaptive T cells. Although ILCs lack somatically recombined TCRs, they share numerous biological features with T cells, most notably their effector functions (Cherrier et al., 2018). Consistently, activated T cells and ILCs are strikingly similar transcriptionally and epigenetically (Shih et al., 2016). Very little is known about the mechanisms controlling these similarities, but the observation that T cell–specific genes such as TCR genes are expressed at early stages of ILC development (Yu et al., 2016) indicates that these similarities start being imprinted very early during development. Transcription factors such as TCF-1, GATA-3, or Bcl11b, which are well appreciated for their critical roles in imprinting T cell identity early during development, are also expressed and required at early stages of ILC development (Kostrzewski and Brady, 2015; Yagi et al., 2014; Yang et al., 2015; Yu et al., 2016). It is tempting to speculate that these shared factors imprint shared epigenetic and transcriptional features on early T cells and ILC precursors, leading to functional similarities between the two lineages at mature stages.

Other previous work, however, indicated that Bcl11b must have some distinct functions in T cells and ILCs. Bcl11b is expressed at commitment to the T cell lineage and is essential to repress ILC fate (Kueh et al., 2016; Li et al., 2010). Mechanistically, Bcl11b directly represses expression of the transcription factor Id2 (Hosokawa et al., 2018) that would otherwise divert T cell precursors toward the ILC lineage (Miyazaki et al., 2017; Wang et al., 2017). In ILCs, Bcl11b is expressed after commitment to the ILC lineage in a subset of ILC precursors destined to become ILC2s. It is then specifically expressed and required in ILC2s (Kostrzewski and Brady, 2015; Yu et al., 2016). On the other hand, Id2 is expressed and required in all ILCs from very early stages of development (Yang et al., 2015; Yokota et al., 1999). Bcl11b and Id2 are thus continuously coexpressed in ILC2s. These observations indicated that Bcl11b does not repress *Id2* in ILC2s, distinct from its actions in T cells.

In this issue of *JEM*, Hosokawa et al. tackled the seemingly conflicting role of Bcl11b in T cells and ILC2s. They characterized T cell–committed precursors and ILC2 lineage cells, epigenetically, transcriptionally, and biochemically, to identify Bcl11b gene targets, protein partners, and post-translational modifications. Using chromatin immunoprecipitation sequencing, the authors found that Bcl11b bound largely distinct genomic regions in T lineage cells and ILC2s, and it regulated almost completely different sets of genes. The authors examined whether Bcl11b partnered with distinct cofactors in the two lineages, which might regulate its binding and function. RUNX1 and RUNX3 directly interacted with Bcl11b and largely colocalized with Bcl11b in both T cell precursors and ILC2s. Analysis of DNA binding motifs enriched at Bcl11b and RUNX binding sites in ILC2s further suggested that a bZIP factor could dictate RUNX and, subsequently, Bcl11b genomic binding in ILC2s. The authors assessed roles for the bZIP factor BATF; however, other bZIP factors, including NFIL3 and BATF3, are expressed early in ILC development and may also impose differences in the regulatory landscape between T cell and ILC precursors before Bcl11b expression. Interestingly, mass spectrometry indicated that Bcl11b presented differential post-translational modifications in T cells and ILCs that might also result in lineage-specific functions.



Insights from Christelle Harly and Avinash Bhandoola.

Hosokawa et al. (2019) further examined how *Bcl11b* expression is activated at ILC2 commitment. They used a *Bcl11b*-reporter mouse in which a distal enhancer important for *Bcl11b* expression in T cells was deleted (Kueh et al., 2016). They found that this enhancer also contributed to *Bcl11b* expression in ILC2s. Interestingly, assay for transposase-accessible chromatin using sequencing (ATAC-seq) data showed that this enhancer was inactive in mature ILC2s, indicating that the factors controlling it acted at earlier stages of development. Consistently, several factors that regulate *Bcl11b* expression through this enhancer in T cells (Kueh et al., 2016) are also expressed during early ILC development (Harly et al., 2019). These include PU.1, RUNX1, TCF-1, and GATA-3, which are expressed during early stages of T cell and ILC development before *Bcl11b* expression. The authors' work thus

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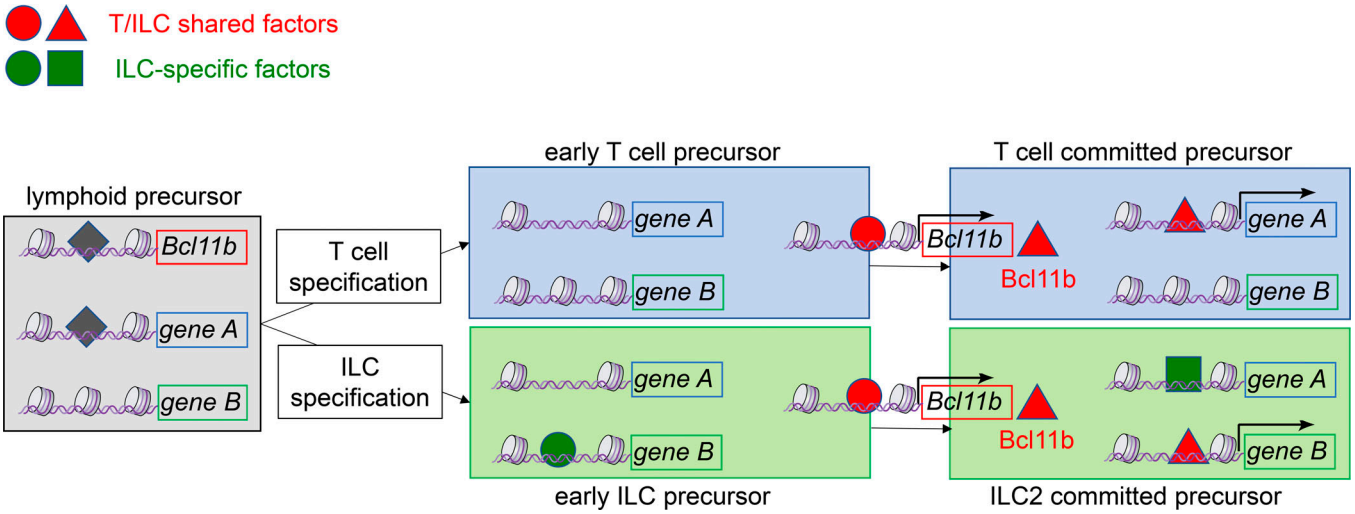

Transcription factors (such as PU.1 and RUNX1) expressed at common progenitor stages establish an epigenetic and transcriptional landscape largely shared by early T cell precursors and ILC precursors. Transcription factors up-regulated in both early T cell and ILC precursors (TCF-1, GATA-3) use this shared landscape and regulate shared gene targets in the two lineages that include *Bcl11b*. Transcription factors active in ILC precursors (including a putative bZIP factor) additionally impose lineage-specific features at the epigenetic and transcriptional levels. These early differences result in distinct functions for Bcl11b. Shared factors (genes and proteins) are shown in red, and ILC specific factors in green.

indicates that transcription factors that are shared by early T cells and ILC precursors clearly do play some shared functions in the two lineages, including activation of *Bcl11b* expression, in line with other recent work examining TCF-1 function in T cell and ILC lineages (Harly et al., 2019). Shared functions of these early shared transcription factors upstream of Bcl11b may depend on epigenetic and transcriptional landscapes inherited from common progenitor stages.

Future work should investigate how the multiple levels of regulation of Bcl11b function are layered during early ILC development. These studies will help understand how Bcl11b function is controlled by lineage-specific factors that modulate its binding or activity (e.g., ILC-specific binding partners or post-translational modifiers). The putative ILC-specific bZIP factors identified by the authors that could dictate RUNX and Bcl11b binding are of particular interest to understand how Bcl11b plays distinct functions in T cells and ILCs. How such factors come to be expressed in ILC precursors but not T cells is another critical question. Importantly, many transcription factors, both shared (PU.1, RUNX, TCF-1) and lineage specific (PLZF, NFIL3, BATF3), expressed during early ILC development are down-regulated before Bcl11b expression (Harly et al., 2019). Thus, although these factors might not directly interact with Bcl11b to control its binding location or functional activity, they may together establish a distinct epigenetic and transcriptional landscape for Bcl11b and cofactors to act at later stages of ILC development.

In summary, Hosokawa et al. (2019) collected an astonishing amount of information on ILC2s and T cell precursors to document multiple levels of regulation of Bcl11b. As a result, Bcl11b is involved in the regulation of almost completely distinct gene targets at the establishment of T cell identity, and in ILC2s. The work highlights the difficulties in predicting transcription factor functions and the necessity to integrate complementary epigenetic, transcriptomic, and proteomic approaches. A dynamic perspective is further required to understand how several levels of regulation can be layered during development and lead to lineage-specific function of transcription factors. Continuing technological advances (Shema et al., 2019) that allow study of rare developmental intermediates such as ILC precursors will enable further discoveries of mechanisms underlying the establishment of cellular identity during development.

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