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Editorial: **GCN5 opens the door for the IRF-4-mediated cascade of B cell differentiation**

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B cell development is a multilayered process that involves the restructuring of the transcriptional landscape of progenitor cells. As B cells progress through differentiation, they pass through discrete stages, during which, alternative lineage potential is lost, and a mature phenotype is acquired. The sequential orchestration of shifting cellular transcriptomes during this process is delicately controlled. Whereas the presence of transcriptional activators and repressors are important players in genetic programming during development, their functions are intertwined with and dependent on the chromatin configuration of the genes that they control. Transcriptome analysis has enabled many groups to determine important gene sets present in developing and mature subsets, including those of the T and B cell lineages [1]. However, to fully elucidate the gene regulatory networks of cells of a specific lineage at a defined developmental stage, the status of DNA occupancy must be assessed, in addition to the levels of mRNA for each regulator-target gene pair. Furthermore, the histone signatures that mark "open", "closed", and "poised" chromatin configurations are important for understanding how genes change over developmental time, even before mRNA expression commences. Thus, assessing the epigenetic regulation of gene expression during B cell development will be critical for a full picture of how these processes are regulated.

Here, Kikuchi et al. [2] show that the HAT, GCN5, is directly linked to transcriptional control of IRF-4, an important regulator of B cell development. The widespread expression and functions of HATs in development have made it challenging to study these genes in a loss-of-function approach in specific cell types. Kikuchi et al. [2] have therefore taken the clever approach of creating a somatic GCN5 germline deletion (GCN5−/−) in the chicken immature B cell line, DT40. First, they used a quantitative RT-PCR approach to survey these cells for expression of known regulators of B cell development and showed that cells lacking GCN5 showed a significant reduction in the expression of IRF-4 and Blimp-1. Next, they were also able to show by reconstitution studies that forced IRF-4 expression could rescue Blimp-1 expression in GCN5−/− cells, consistent with the known role for IRF-4 in direct up-regulation of Blimp-1 in developing B cells [3]. Blimp-1 reconstitution of GCN5−/− DT40 cells, however, failed to restore IRF-4 expression, suggesting that Blimp-1 does not regulate IRF-4 or that regulation of IRF-4 by Blimp-1 is GCN5-dependent. Importantly, ChIP analysis showed that GCN5 binds to and acetylates the histones within the 5′ region of IRF-4, indicating a direct mode of regulation. GCN5 was incapable, however, of binding to the upstream promoter region of Blimp-1, indicating that the loss of Blimp-1 was mediated primarily by the disruption in IRF-4 expression. These results elegantly show that epigenetic regulation of IRF-4 is critical for the maintenance of a genomic landscape compatible with B cell differentiation.

Numerous studies have begun to unravel the epigenetic regulation of genes during different stages of B cell development, but the way in which chromatin remodeling factors are targeted to specific genes in distinct cell types is still largely unknown. It has been shown recently that the accessibility of transcription factor genes important for the pro-B cell stage, such as EBF-1, can be regulated based on their presence in specific genome "domains", which specify transcriptionally permissive or repressive states [4]. EBF-1 itself has the ability to infiltrate chromatin at its target genes to open up the locus, thus acting as a "pioneer" factor [5]. On the other hand, Blimp-1 has been shown to interact with H3 methyltransferase G9a [6] and histone deacetylase 1 and 2 [7], both of which aid in transcriptional silencing. The results of Kikuchi et al. [2] therefore suggest a cascade of events that begins with the GCN5-dependent upregulation of IRF-4, followed by GCN5-independent induction and activity of Blimp-1, which then silences a suite of genes that restrain plasma cell development, including Bcl6 and Bach2 (**Fig. 1**). How GCN5 is targeted to IRF-4 in the DT40 cells and whether that targeting is also found in primary B cells, however, remain to be determined.

One gene that was decreased in expression in GCN5−/− DT40 cells and not restored by IRF-4 or Blimp-1 was Ikaros, which associates with nucleosome remodeling deacetylase complexes and participates in chromatin remodeling but is also instrumental in the up-regulation of genes critical for lymphoid lineages [9]. However, the results reported here suggest that expression of Ikaros itself may be GCN5-dependent (Fig. 1). Other studies have shown that Ikaros expression is HAT-dependent under some conditions [10], consistent with this hypothesis. Interestingly, GCN5 can also acetylate transcription factors themselves and influence their activity directly. For instance, GCN5 has been shown to interact directly with and acetylate the E2A-Pbx oncogenic fusion protein, resulting in increased protein stability [11]. This raises the possibility of developing GCN5-specific inhibitors to destabilize E2A-Pbx in B cell leukemias and emphasizes the importance of understanding the impact of disruption in GCN5 activity on immature B cells on a wider scale. If GCN5 interacts with the WT form of E2A in a similar manner, then the loss of GCN5 in DT40 cells might be expected to result in a decrease in E2A protein levels or activity that would not be detectable at the mRNA level. Thus, there are many layers of regulation that are impinged on when removing GCN5 that should be considered.

Although these results provide important information about the regulation of IRF-4 and Blimp-1 in the context of an

Abbreviations: Bcl6=B cell lymphoma 6, Blimp-1=B lymphocyte-induced maturation protein 1, ChIP=chromatin immunoprecipitation, EBF-1=early B cell factor 1, GCN5−/−=GCN5-deficient, HAT=histone acetyltransferase, IRF-4=IFN regulatory factor 4, Pbx=pre-B cell leukemia homeobox 1, seq=sequencing, WT=wild-type

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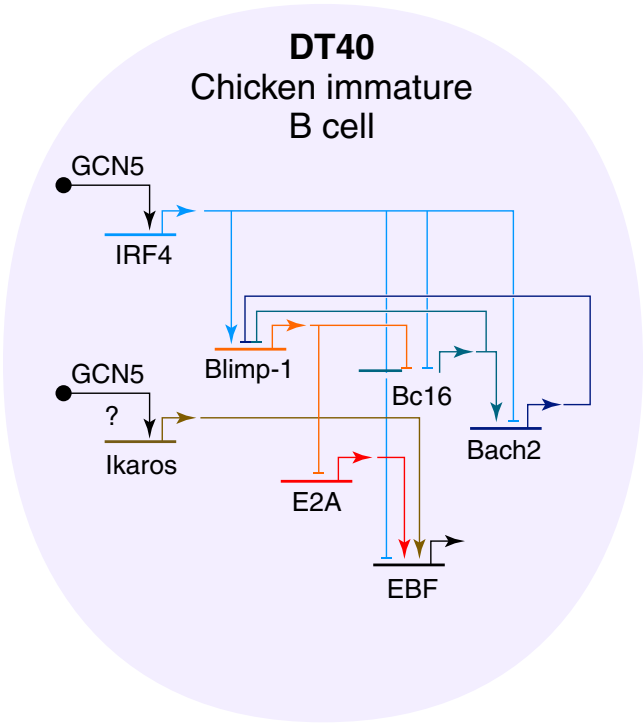

Figure 1. Hypothetical gene regulatory network downstream of GCN5 in DT40 B cells (assembled based on Supplemental Fig. 1 of Kikuchi et al. [2] and ref. [8]). Blimp-1 interacts antagonistically with Bcl6 and Bach2 to control the plasma cell differentiation process, whereas the effects of IRF-4 on this process are level-dependent. Here, the inputs of proposed GCN5-dependent events are depicted. GCN5 is required for the induction of IRF-4 and acts directly on its promoter. GCN5−/− DT40 cells exhibit up-regulation of Bach2, Bcl6, E2A, and EBF relative to WT DT40 cells. IRF-4 reconstitution of GCN5−/− DT40 cells leads to an upregulation of Blimp-1 and downregulation of Bcl6, Bach2, E2A, and EBF. In the absence of GCN5 and IRF-4, Blimp-1 fails to repress Bach2 or EBF-1, suggesting that these regulatory events require direct inputs from IRF-4. Blimp-1 is capable of restoring Bcl6 and E2A levels in GCN5−/− DT40 cells in the absence of IRF-4, suggesting direct inputs. Ikaros is lost in GCN5−/− cells and fails to be restored with either Blimp-1 or IRF-4, suggesting that direct regulation by GCN5 of Ikaros is necessary in this context.

immature B cell-like environment, there are several caveats to be considered. DT40 cell lines provide an excellent model for interrogating gene expression changes within a snapshot stage of B cell development. However, they are not undergoing development under steady-state conditions and thus, are not following the waves of transcriptional change needed to progress from one stage to another. Secondly, they are cultured outside of the inductive environment in which B cell development normally takes place and therefore, in a different signaling milieu. Interestingly, in vivo experiments suggest that Blimp-1 and IRF-4 cross-regulate each other during the transition from effector B cell to plasma cell [8], whereas there is no evidence of Blimp-1 induction of IRF-4 in the DT40 system. However, GCN5−/− undoubtedly impacts many genes, some of which may be required to collaborate with Blimp-1 to modulate IRF-4 expression. One of the advantages of cell lines is the ability to generate large numbers of cells that can be used in more comprehensive strategies, such as RNA-seq and ChIP-seq analyses. These would provide a much more complete picture of how this chromatin remodeling factor impacts the transcriptional landscape of effector B cells in a way that would be very difficult to achieve in primary cells. However, although global investigations provide vast and comprehensive data sets, it is imperative to confirm individual protein interactions and mechanisms of gene regulation, which Kikuchi et al. [2] have reported here with GCN5, IRF-4, and Blimp-1.

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