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A synchronous IRF4-dependent gene regulatory network in B and Th cellsorchestrating the antibody response

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Abstract

Control of diverse pathogens requires an adaptive antibody response, dependent on cellular division of labor to allocate antigen dependent B and $CD4^+$ T cell fates that collaborate to control the quantity and quality of antibody. This is orchestrated by the dynamic action of key transcriptional regulators mediating gene expression programs in response to pathogen-specific environmental inputs. We describe a conserved, likely ancient, gene regulatory network that intriguingly operates contemporaneously in B and $CD4^+$ T cells to control their cell fate dynamics and thus, the character of the antibody response. The remarkable output of this network derives from graded expression, designated by antigen receptor signal strength, of a pivotal transcription factor that regulates alternate cell fate choices.

Keywords

Transcription factors; antibody response; Blimp-1; Bcl6; IRF4; cell differentiation

Diverse antibody production requires both B and $CD4^+$ T cells in vertebrates

Upon antigen-dependent activation of the B cell receptor (**BCR**, see glossary) and supportive signals from $CD4^+$ T cells, B cells differentiate into cells capable of BCR diversification by somatic hypermutation (**SHM**) and/or class switch recombination (**CSR**), **antibody** secretion, or long-term memory [1] (Figure 1). Each of these possible cell fates are governed by state-specific gene programs and associated transcriptional regulators. For example, **germinal center** B cells (**GCB**s) are dependent on the transcription factor (TF) **Bcl6** for differentiation, and on the activity of the enzyme Activation-Induced Deaminase (**AID**) for SHM and CSR [2]; by contrast, antibody secreting plasma cells (PCs) depend on the activation of TF Blimp-1 for their terminal differentiation [3], [4]. Thus, distinct cell types execute antibody quantity (secretion) and quality (a product of diversification).

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Antigen engagement of the T cell receptor (**TCR**) drives the differentiation of $CD4^+$ T cells that orchestrate an appropriate immune response [5]. Similar to B cells, $CD4^+$ T cell fate choices are dictated by distinctive gene programs and transcriptional regulators [6]. $CD4^+$ T follicular helper (**Tfh**) cells, dependent on the TF Bcl6, provide help to B cells in the form of survival, activation, and regulatory signals during the process of **affinity maturation** [7]–[9]. In contrast, $CD4^+$ T effector (**Teff**) cells, dependent on the transcription factor Blimp-1, migrate to peripheral tissues to participate in local immune processes [10]–[13]. Thus, the antibody response is dependent on the proportional allocation of Tfh cells, which is in turn regulated by the relative expression of Bcl6 and Blimp-1 [14].

Bcl6 and Blimp-1 act as counter-antagonists to mediate cell fate allocation. Findings that the TF **IRF4** was both essential and upstream of the Bcl6 and Blimp-1 cell fate determinants in both B and $CD4^+$ T cells, prompted the formation of a novel model for how cellular concentrations of IRF4 quantitatively and qualitatively might tune the antibody response [15]–[18]. Herein, we describe the genetic and signaling perturbations that led to the description of a primary mechanism of B and $CD4^+$ T cell alternate cell fate choice. We provide a molecular view for how B and $CD4^+$ T cells integrate IRF4 cell concentrations and assemble a core gene regulatory network (Box 1) that explains the dynamics of the network (Box 2) in orchestrating alternate cell fate choice and ultimately, the nature of the antibody response (Figure 2, Key Figure). Altogether, a larger model emerges whereby IRF4, operating contemporaneously in B and $CD4^+$ T cells, may enable the collaboration of specialized antigen specific B and $CD4^+$ T cells to regulate the makeup of the mammalian antibody response [15], [16]. The information in this review pertains to experiments conducted either on mouse and/or human cell lines, and/or primary mouse and/or human cells.

Core components of the B and $CD4^+$ T cell IRF4 Gene Regulatory Network

The antigen receptor: BCR and TCR

Upon antigen binding by antigen receptors, a “signalosome” is assembled that recruits a variety of intermediates which branch to activate multiple second messenger signaling cascades [19]. The *Irf4* locus in mammals is an immediate early gene induced by antigen receptor signaling in both B and $CD4^+$ T cells [20], specifically through the downstream effectors NF-kB, NFAT, and mTOR [21]–[23]. Thus, antigen receptor signaling activates multiple second messenger cascades that lead to broad cellular activation, and which also converge on the *Irf4* locus. Although IRF4 cellular concentrations are important for B and $CD4^+$ T cell differentiation, the basis for, and whether the converging pathways are additive, synergistic, or subject to Boolean logic, remains to be explored.

$CD4^+$ T and B cell differentiation is affected by both the biophysical properties of antigen recognition and subsequent signaling intensity [24], [25]. High signal strength in B cells enhances the proportions of effector, antibody secreting PCs, whereas low signal strength favors antibody diversification through CSR in the germinal center (GC) [26]–[31]. In $CD4^+$ T cells, greater antigen receptor signal intensity favors the formation of inflammatory effector $CD4^+$ T cells (Teff) cells, whereas lower signal strength favors differentiated cells that function to help B cells [15], [32]–[34]. While the “signalsome” is common to both

strong and weak antigen receptor signaling, it is a matter of current research to identify the nature of the receptor signal strength that regulates cell fate allocation during the immune response.

Transcription factor IRF4

The Interferon Regulatory Family (IRF) includes a group of TFs involved in signaling pathways essential for multiple cellular processes, including cell growth and immune regulation [35]. Nine human IRF genes have been identified that all share significant homology in their DNA-binding domains, comprised of five tryptophan repeats, three of which contact at DNA sequences termed Interferon-Sensitive Response Elements (**ISRE**) [36], [37]. The carboxy terminus includes the IRF associated domain (IAD), which displays greater sequence diversity and has been linked to binding partner specificity and autoregulation of DNA binding [35]. Unlike all other IRFs, IRF4 is not regulated by Type I or Type II interferons (IFN) [20], but is instead activated by antigen receptor signaling and restricted classes of Toll-like receptors (**TLR**) (TLR4 or TLR9) and Tumor Necrosis Factor Receptor (CD40), suggesting a unique role for IRF4 [17], [38]–[40].

. IRF4 DNA binding has been shown to occur at multiple sites, which depend on distinct partner requirements and composite sequence elements (Table 1). For example, biochemical analysis of the immunoglobulin light chain enhancer region showed that IRF4 is in a ternary complex with either PU.1 or SpiB (both of the Ets family) and Ets Irf Composite Elements (**EICE**) [41]. Biochemical analysis of the ternary complex revealed that the Ets proteins recruit IRF4 to enable high affinity IRF4 binding, whereas binding by IRF4 alone was extremely inefficient [42]. In addition, IRF4 partners in a quaternary complex with BATF/c-jun or BATF/JunB heterodimers (AP-1 family members) and AP-1 IRF Composite Elements (**AICE**) [16], [43]–[46]. Indeed, biochemical experiments using the AICE probe sequence have revealed a similar partner dependency for high affinity binding, as described for EICE[41]. Further, two variants of AICE sequences display slightly different dependencies on IRF4 concentrations for efficient binding suggesting even greater complexity to the generalizations in this review [43], [47]. Finally, chromatin immunoprecipitation sequencing (ChIP-seq) studies in activated B cells have highlighted IRF4 bound regions, including the *Prdm1* (Blimp-1) locus, that contain multiple copies of the ISRE sequence [16], [48]. However, ISRE binding by IRF4 has been shown to be of comparatively lower affinity to that of either EICE or AICE [15], [16], [49]. Thus, a general biochemical scenario emerges whereby IRF4 displays relatively higher affinity DNA binding when it is recruited by Ets or AP-1 members to EICE or AICE composite elements, respectively, but comparatively poorer DNA binding efficiency to ISRE multimers. As we discuss below, these properties of differential DNA affinity likely enable B and $CD4^+$ T cells to decode graded IRF4 cell concentrations set by antigen receptor signal strength to execute alternate cell fate decisions.

A number of other composite sequences and binding partners have also been identified in B and T cells, including NFAT, E2A and Ikaros [50]–[52]. In relation to NFAT and Stat3, these TFs are often found to co-bind with IRF4 to cis-regulatory elements in genome-wide experiments; however, whether this reflects cooperative assembly on composite elements remains to be determined[53], [54]. Thus, the regulation of IRF4 genome binding is highly

complex, suggesting that the dynamics of IRF4 DNA binding and partner interactions may underlie the differential activity of IRF4.

The phenotype of Irf4 deficient mice was first described in 1996, where the formation of the bone marrow and splenic naïve B and T cell compartments appeared normal; however, all tested antigen dependent responses were compromised [38]. Since then, seminal work from many laboratories demonstrates that IRF4 plays a broad role in B and T cell responses, and furthermore, suggests that, unlike lineage-specifying TFs (e.g. Tbet, Rorc), IRF4 plays a novel and fundamental role in the activation and differentiation of multiple subsets of B and T cells [18], [23], [60]–[69], [38], [70], [71], [52], [53], [55]–[59]. Cumulatively, these important findings prompted us to pursue a distinct line of reasoning regarding the role of IRF4 in T cell differentiation. We describe this below in the context of the T-dependent antibody response.

Transcription factor Bcl6

B cell lymphoma 6 (*Bcl6)* was first identified as a potential proto-oncogene from a chromosome translocation frequently seen in diffuse large B-cell lymphoma [72] and was later shown in cell lines to be involved in apoptosis regulation [73]–[75]. Bcl6 is a zinc-finger TF that also bears a POZ (or BTB or ZIN) domain in the amino terminus [72], [76]; this domain is primarily responsible for transcriptional repression via recruitment of corepressor proteins that include histone deacetylases[76]–[78]. *Bcl6* is highly expressed in GCBs; $Bcl6^{-/-}$ mice are impaired in GCB differentiation, and mice which constitutively express *Bcl6* in B cells have enhanced GCB responses relative to wild type (WT) mice [79]–[81]. Furthermore, Bcl6 has also been shown to be both necessary and sufficient for Tfh differentiation in mice [7]–[9]. Therefore, transcriptional repression by Bcl6 is key to adopting GCB and Tfh cell fates, in large part through the suppression of alternative cell fate determinants (including Blimp-1) [14], [82].

Transcription factor Blimp-1

B lymphocyte induced maturation protein-1 (Blimp-1; encoded by *Prdm1*) is a zinc finger TF that functions as both a repressor and activator of gene expression [83]–[85]. Blimp-1 contains five Krüppel-type zinc finger motifs, a proline-rich domain implicated in gene repression, and a SET domain that functions to control antibody secretion [84], [86], [87]. Blimp-1 was first described in murine B cells as a gene highly induced during B-cell differentiation into PCs, and was later shown to be both necessary and sufficient for PC differentiation [86], [88]. In PCs, Blimp-1 regulates a large gene program important for high titer antibody secretion –operating at both the post-transcriptional and post-translational levels [83]–[85]. Subsequently, Blimp-1 was shown to play an essential role in effector T cell (Teff) differentiation and the acquisition of migratory, cytolytic, and inflammatory gene programs [12], [13], [61]. In addition to regulating a gene program important for PC and Teff cell function, Blimp-1 can also repress the expression of Bcl6 –thus forming a counter-antagonistic transcriptional regulatory loop [7], [82], [84]. Thus, reciprocal repression of Blimp-1 and Bcl6 is believed to be at the core of the mechanism enabling divergent cell fate choices for B and $CD4^+$ T cells.

The B and CD4+ T cell IRF4 Gene Regulatory Network

IRF4 induces both Blimp-1 and Bcl6 expression

The essential role of IRF4 in the antibody response has been known for over 20 years, when immunized *Irf4*$^{-/-}$ mice failed to produce both antigen-specific serum antibody, and GCBs [38]. Subsequent *ex vivo* experiments using these same *Irf4*$^{-/-}$B cells demonstrated that PC differentiation depended on IRF4 induction of Blimp-1 in a cell autonomous manner [17]. These studies also revealed that IRF4 was essential for the expression of AID, which placed IRF4 in a network upstream of essential regulators of B cell differentiation. IRF4 was later shown to be essential for cell autonomous upregulation of Bcl6 and the generation of GCBs, but timing was important as this was only observed when IRF4 was deleted prior to antigen activation and not post antigen activation (using CD19- or Cγ1- Cre drivers, respectively) [16], [18], [39]. The timing of deletion exhibited by the different Cre drivers suggested that IRF4-dependent GCB cell fate decisions occured in the first few days following antigen encounter in mice.

Simultaneous with B cell studies, Tfh cells required the expression of Bcl6 and were antagonized by Blimp-1 in gain- and loss-of-function experiments [7]. However, the factor(s) responsible for activating Bcl6 and/or Blimp-1 cell fate determinants in CD4^{+} T cells were unknown. Later, it was demonstrated that *Irf4*$^{-/-}$ mice failed to generate Tfh cells [53]. A cell autonomous role of IRF4 in generating Tfh cells was confirmed using bone marrow chimeras; and this study also established that *Irf4*$^{-/-}$ CD4^{+} T cells failed to generate Blimp-1-expressing Teff cells that co-expressed *Tbx21* (T-bet) and secreted proinflammatory IFNγ[15]. Together, these results demonstrated that IRF4 was central to both diversification and effector cell fates in both B and CD4^{+} T cells, positioned upstream of the key cell fate determinants Blimp-1 and Bcl6 (Figures 3 and 4).

IRF4 Expression dictates Blimp-1 or Bcl6 gene induction

The data described above raised the question, “how can IRF4 simultaneously activate the expression of two counter-antagonistic transcriptional regulators”? Insight arose with the observation that PCs were restricted to high IRF4 expression, whereas GCBs, to low IRF4 expression [17], [89]. This raised the possibility that IRF4 cell concentrations could coordinate the cell fate decision between Blimp-1-expressing PC and Bcl6-expressing GCB. Genetic perturbation experiments were consistent with this idea [17], [40], [90], but strong evidence came from the analysis of a mouse germline tetracycline-inducible allele of *Irf4*, which enabled orthogonal control of IRF4 expression *in vitro* and *in vivo* in antigen specific B cells (uncoupled from BCR/TCR) [16]. This system showed that low, transient amounts of IRF4 could rescue GCB differentiation but were insufficient for PC differentiation. Conversely, when IRF4 expression was enforced above WT levels, PC differentiation was enhanced at the expense of the GCB response. To formally test whether IRF4 abundance directed CD4^{+} T cell fate determination, the same mouse germline encoded *Irf4*-inducible system in antigen specific TCR transgenic cells was used *in vivo* [15]. Enforced induction of IRF4 above WT, resulted in a substantial redirection of Tfh to Teff cell fates. This tetracycline-inducible IRF4 system demonstrated that despite the multiple pathways induced

by antigen receptor signaling, the pathway controlling IRF4 induction appeared to be key for B and $CD4^+$ T cell fate choices [15].

IRF4 expression scales with antigen receptor signaling intensity

Due to *Irf4* being an immediate early gene downstream of the BCR, it was reasoned that BCR signal strength might regulate IRF4 expression amounts. Indeed, stimulating antigen-specific B cells *in vitro* withincreased antigen abundance or avidity raised IRF4 cell concentrations in a manner that scaled with signal intensity [40]. Moreover, using the **SWHEL transgenic** BCR *in vivo* system, in which low affinity HEL antigens predominantly generate a GCB response and high affinity HEL antigens promotes a PC response[26], IRF4 expression in activated SWHEL B cells was reported to correlate with BCR signal strength [16]. These systems directly linked antigen affinity, and thus, BCR signaling intensity, to PC and GCB cell fate choice via modulation of IRF4 expression. Furthermore, CD40 signaling also induced IRF4 expression, suggesting that B cell engagement with T cells through CD40:CD40L binding could control IRF4 abundance [17], [39], [91]. Because of the temporal delay of CD40-induced versus BCR-induced IRF4 expression, it remains to be determined whether the overall magnitude and/or the timing of IRF4 induction functions as an additional layer of regulation in alternative cell fate choices and thus, in the quantity and quality of the antibody response.

As seen in B cells, the relationship between the antigen receptor and IRF4 expression also occurs in $CD8^+$ T cells [23], [58], [59]. To examine this possibility in $CD4^+$ T cells, an approach was adopted in which a series of **altered peptide ligands (APLs)** were used that displayed a range of potency for the **5c.c7 TCR peptide:MHC system** in mice[25]; the study showed that *Irf4* transcript and protein abundance scaled with increased TCR signal strength both *in vitro* and *in vivo* [15]. These results were consistent with *in vitro* anti-CD3 experiments from another study, which showed that IRF4 protein was induced at higher concentrations with greater TCR stimulation [47]. Together, these observations establish an important link between antigen receptor signal strength and IRF4 abundance.

The *in vivo* relationship between the TCR and IRF4 cell concentrations led to testing whether TCR signal strength influenced Teff and Tfh cell fate decisions. Indeed, upon immunization with low potency APL, responding mouse 5c.c7 TCR transgenic cells of the lymph node exhibited higher frequencies of Tfh cells, coincident with diminished Teff responses relative to high potency APL [15]. This was true at both early and late time points, corresponding to specification and commitment of cell fates, respectively [92]. Conversely, immunization with higher potency APL reversed these trends and favored Teff cell fates at the expense of Tfh [15]. These results confirmed that increased TCR signaling could induce Teff cell fate determination at the expense of Tfh.

The role of TCR signal strength in $CD4^+$ T cell fate choice is well established; however, in relation to Tfh cell fate, whether strong or weak signaling is important remains controversial. Although earlier reports suggested that increased TCR signal strength favored Tfh over Teff cell allocation [93], [94], recent reports in different TCR systems suggested that increased TCR signal strength in $CD4^+$ T cells favored Teff over Tfh cell fates [32]–[34], supporting the data described above. The basis for the divergent conclusions is presently unclear

because of the different TCR systems used, raising the possibility that the range of signal strength along the curve to which a cell is confined to may lead to different interpretations. For example, when interpreting cell fate outcomes stimulated from two weak APLs; the stronger of the weak APLs may lead to comparatively more Tfh because neither APL elicits sufficient TCR signal strength to cross the crucial threshold important for Teff cell choice. We expect future research to identify the biophysical and/or dynamical parameter of TCR signal strength that corresponds to quantitative changes in IRF4 abundance and $CD4^+$ T cell fate outcomes. Lastly, it is clear that IRF4 expression scales with increasing TCR signal strength, and that orthogonally increasing IRF4 is sufficient to derail a given regime of TCR signal strength and its allocation to Tfh and Teff cell fates [15] suggesting that quantifying IRF4 expression in these different systems may resolve the debate.

IRF4 abundance can dictate DNA binding site choice

Previously, the manner in which B cells were thought to detect IRF4 concentrations remained ambiguous, until IRF4 genome localization was measured using Chromatin Immunoprecipitation-deep sequencing (ChIP-seq) [16]. The majority of IRF4 binding events in activated B cells occurred at regions harboring EICE sequences and were precipitated with PU.1. The remaining third of binding events were enriched for either AICE sequences prior to differentiation, or enriched for tandem ISRE sequences upon PC differentiation, demonstrating the complexity in IRF4 binding site choice [16]. In contrast to the EICE, which contains a single ISRE sequence, the tandem ISRE sequences identified in the IRF4-bound regions were found in multiple copies, which raised the possibility that IRF4 could efficiently bind to those sites, perhaps as a dimer or multimer. In fact, biochemical experiments demonstrated specific binding of IRF4 to a tandem ISRE site found in the *Prdm1* (Blimp-1) locus and binding was shown to be considerably weaker when compared to IRF4 binding to an EICE probe in the presence of PU.1 [16]. Thus, at the genome level, IRF4 localized to multiple regions that harbored distinct binding sites, which seemed to differ in their ability to recruit IRF4[16]. To investigate the nature of IRF4 binding with gene regulation and cell fate determination, ChIP-seq datasets were integrated with genome wide expression analysis. IRF4 binding site choice correlated significantly with the binding of genes expressed in cells undergoing different cell fates. Generally, IRF4-bound EICE --but not ISRE sequences--aligned with genes of the GCB program (including *Bcl6*) and complementary to this, IRF4-bound ISRE-- but not EICE sequences--, localized to genes affiliated with the PC program (including *Prdm1*)[16]. Given that ISRE sequences displayed lower IRF4 binding affinity than EICE sequences in the presence of PU.1, this finding suggested that higher amounts of IRF4 were required in PCs to enable efficient occupancy of ISRE-containing regulatory regions of PC genes [16]. Of note, not all binding events in PCs are restricted to low affinity binding sites, as observed for a high affinity IRF4-Ikaros DNA binding complex (ZICE [Zinc finger-IRF Composite Elements]) at select B cell lineage genes, including *Ebf1*, known to antagonize PC differentiation [51]. Together, a model emerges whereby B cell fate choice, and therefore the character of the antibody response, are shaped by a given cell's IRF4 abundance, in turn dictating IRF4 binding partner interactions, and the ability of this TF to occupy low affinity DNA binding sites.

To better understand how IRF4 abundance molecularly controls $CD4^+$ T cell fate choices, the relationships between accessible chromatin (putative regulatory regions) have been analyzed in $CD4^+$ T cells with differential gene expression by ATAC-seq, along with the presence of low or high affinity IRF4 DNA binding sites [15]. Cells depleted of IRF4 did not express genes of either the Tfh or Teff program, cells with WT amounts of IRF4 expressed genes of either the Tfh or Teff programs and these cells had greater accessibility to Tfh program genes, including Bcl6. Moreover, cells with abundant IRF4 predominantly expressed, and had greater accessibility to genes of the Teff program, including *Prdm1*, while exhibiting a collapse of the Tfh program. Low affinity ISRE sequences were significantly enriched in regions belonging to the Teff program and depleted in regions aligned with the Tfh program. Conversely, high affinity AICE sites were enriched in regulatory regions of both the Tfh and Teff programs [15]. Hence, high affinity AICE sequences might also regulate Teff genes, which suggests that as IRF4 accumulates, transient binding to AICE sequences might "prime" Teff-associated loci for later expression during Teff cell commitment. Thus, as in B cells, the dynamics of IRF4 accumulation enable differential occupancy of high and low affinity DNA binding sites of genes belonging to the Tfh and Teff programs to control cell fate allocation. Nevertheless, further analyses are warranted to fully validate these models, and more could be done by using mathematical interpretations (Box 3).

Concluding Remarks

There is genetic evidence for the IRF4 gene regulatory network (GRN) in specifying antigen dependent B and $CD4^+$ T cell fate trajectories that orchestrate the antibody response. However, it is important to note that the IRF4 GRN is a core network and thus, it is expected that signal-induced pathways activate other GRNs that interface directly or indirectly with it to modulate the overall dynamics depending on the output of those GRNs [121]-[122]. In fact, recent computational modeling and wet lab experimental validation with B cells suggest that a reciprocally antagonistic NFκB GRN composed of RelA and c-Rel can interface with the IRF4 GRN on the Blimp-1 node in an antagonistic manner, dampening PC differentiation [123]. In contrast to these deterministic GRN models, an expanding literature of modeling and single cell visualization experiments suggest that B and $CD4^+$ T cell fate trajectories are subject to stochasticity [124], [125]. In light of the inherent "noise" of transcription and translation rates that have been documented using high throughput methodologies [126], it is not surprising that randomness is observed. Thus, a fascinating scenario emerges where deterministic and stochastic events combine to regulate clonal selection and fate allocation to enable the micro-evolution of the antigen-induced repertoire of diversified B and $CD4^+$ T cells [127].

Given that the IRF4 GRN is rooted in TF biology, it is subject to regulation by the nuclear chromatin environment that includes nuclear position and looping as well as epigenetic modifications that act at specific loci. Of note, PCs undergo dynamic de- and pro-DNA methylation events that may enable PC differentiation [128]. How this is regulated and impacts the dynamics of the IRF4 GRN will be exciting to unravel. Another interesting finding is that IRF4 activity has been linked to the regulation of genes important for metabolism [58], [117]. Metabolic changes are key to cell activation and division, but

different cell states and their transitions exhibit distinct metabolic requirements –including GCB, PC, Tfh, and Teff [129]-[133]. The role of IRF4 in metabolic processes and how metabolites may feedback on the IRF4 GRN remains for future studies but is certainly timely. Overall, the core IRF4 GRN both influences and interfaces with a multitude of regulatory processes comprised of gene and signaling regulatory modules to control the outcome of B and $CD4^+$ T cell fate allocation.

In sum, the essential components of a dynamic IRF4 GRN that regulate alternate B and $CD4^+$ T cell fates have been identified, but further work is required to map the ancillary factors, GRNs, and metabolic effectors that integrate the quantity and quality of antibodies in the immune response. Given the large numbers of nodes involved, it is clear that modeling will play an important role in clarifying the nature of these interactions and how they affect the dynamics of the overall process (see Outstanding Questions). The existing and future endeavors in this area promise to advance the fundamental understanding of cell fate determination and provide novel inroads for potential therapeutic targeting

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Glossary

5c.c7 system
A well characterized T cell receptor that recognizes a peptide derived from moth cytochrome c in the context of MHC II I-E^k. Variants of this peptide, called **Altered Peptide Ligands (APL)**, have been identified that display weak and strong agonistic as well as antagonistic activities. These variants reflect TCR contact residues and not MHC contact residues that would attenuate general peptide:MHC density. A genetically modified mouse model based on this TCR enables antigen specific T cells to be tracked in response to the APLs in vivo

B cell receptor (BCR) / T cell receptor (TCR)
The antigen receptor expressed by B and T cells formed by assembling Variable and Joining segments by DNA rearrangements during their development. The receptor recognizes antigen

Germinal Center B cell (GCB)
A B cell state within a germinal center in a lymphoid follicle; it is acquired upon antigen encounter and T cell-derived signals with a genetic program for the diversification of the antigen specific repertoire by somatic mutation of its immunoglobulin variable genes. Selected variants differentiate into plasma and memory B cells

Plasma cell (PC)
A B cell state acquired upon antigen encounter and T cell-derived signals with a genetic program for antibody secretion thus maintaining serum immunoglobulin titers

SWHEL system

A genetically modified mouse model that enables antigen specific B cells to be tracked in response to an allelic series of antigens that vary in affinity to the antigen receptor

T follicular helper cell (Tfh)
A T cell state acquired upon antigen encounter and B cell derived signals with a genetic program for the coordination of B cell activation and differentiation

T effector cell (Teff)
A T cell state acquired upon antigen encounter with a genetic program for the coordination of inflammatory responses at peripheral tissue sites

T-dependent antibody responses
Antigen-specific antibody derived by obligate interactions between antigen specific B and $CD4^+$ T cells. The two-way interactions lead to B and T cell differentiation into the GCB, PC, and Tfh cell states described in this review

Toll Like Receptor (TLR)
A system of innate immune receptors that signal inflammatory responses upon recognition of common microbial patterns. Signaling is often through the NFκB system

References

[1]. Boothby MR, Hodges E, and Thomas JW, "Molecular regulation of peripheral B cells and their progeny in immunity," Genes Dev., vol. 33, no. 1–2, pp. 26–48, 01 2019, doi: 10.1101/gad.320192.118. [PubMed: 30602439]

[2]. Song S. and Matthias PD, "The Transcriptional Regulation of Germinal Center Formation," Front. Immunol, vol. 9, p. 2026, 2018, doi: 10.3389/fimmu.2018.02026. [PubMed: 30233601]

[3]. Bortnick A. and Murre C, "Cellular and chromatin dynamics of antibody-secreting plasma cells," Wiley Interdiscip. Rev. Dev. Biol, vol. 5, no. 2, pp. 136–149, 4. 2016, doi: 10.1002/wdev.213. [PubMed: 26488117]

[4]. Tellier J. and Nutt SL, "Plasma cells: The programming of an antibody-secreting machine," Eur. J. Immunol, vol. 49, no. 1, pp. 30–37, 2019, doi: 10.1002/eji.201847517. [PubMed: 30273443]

[5]. Ballesteros-Tato A. and Randall TD, "Priming of T follicular helper cells by dendritic cells," Immunol. Cell Biol, vol. 92, no. 1, pp. 22–27, 1. 2014, doi: 10.1038/icb.2013.62. [PubMed: 24145854]

[6]. Vahedi G, Kanno Y, Sartorelli V, and O'Shea JJ, "Transcription factors and CD4 T cells seeking identity: masters, minions, setters and spikers," Immunology, vol. 139, no. 3, pp. 294–298, 7. 2013, doi: 10.1111/imm.12113. [PubMed: 23586907]

[7]. Johnston RJ et al. , "Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation," Science, vol. 325, no. 5943, pp. 1006–1010, 8. 2009, doi: 10.1126/science.1175870. [PubMed: 19608860]

[8]. Yu D. et al. , "The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment," Immunity, vol. 31, no. 3, pp. 457–468, 9. 2009, doi: 10.1016/j.immuni.2009.07.002. [PubMed: 19631565]

[9]. Nurieva RI et al. , "Bcl6 mediates the development of T follicular helper cells," Science, vol. 325, no. 5943, pp. 1001–1005, 8. 2009, doi: 10.1126/science.1176676. [PubMed: 19628815]

[10]. Mackay LK et al. , "Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes," Science, vol. 352, no. 6284, pp. 459–463, 4. 2016, doi: 10.1126/science.aad2035. [PubMed: 27102484]

[11]. Zundler S. et al. , “Hobit- and Blimp-1-driven CD4+ tissue-resident memory T cells control chronic intestinal inflammation,” Nat. Immunol, vol. 20, no. 3, pp. 288–300, 2019, doi: 10.1038/s41590-018-0298-5. [PubMed: 30692620]

[12]. Marshall NB et al. , “NKG2C/E Marks the Unique Cytotoxic CD4 T Cell Subset, ThCTL, Generated by Influenza Infection,” J. Immunol. Baltim. Md 1950, vol. 198, no. 3, pp. 1142–1155, 01 2017, doi: 10.4049/jimmunol.1601297.

[13]. Kallies A, Xin A, Belz GT, and Nutt SL, “Blimp-1 transcription factor is required for the differentiation of effector CD8(+) T cells and memory responses,” Immunity, vol. 31, no. 2, pp. 283–295, 8. 2009, doi: 10.1016/j.immuni.2009.06.021. [PubMed: 19664942]

[14]. Crotty S, Johnston RJ, and Schoenberger SP, “Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation,” Nat. Immunol, vol. 11, no. 2, pp. 114–120, 2. 2010, doi: 10.1038/ni.1837. [PubMed: 20084069]

[15]. Krishnamoorthy V. et al. , “The IRF4 Gene Regulatory Module Functions as a Read-Write Integrator to Dynamically Coordinate T Helper Cell Fate,” Immunity, vol. 47, no. 3, pp. 481–497.e7, 19 2017, doi: 10.1016/j.immuni.2017.09.001. [PubMed: 28930660]

[16]. Ochiai K. et al. , “Transcriptional regulation of germinal center B and plasma cell fates by dynamical control of IRF4,” Immunity, vol. 38, no. 5, pp. 918–929, 5 2013, doi: 10.1016/j.immuni.2013.04.009. [PubMed: 23684984]

[17]. Sciammas R, Shaffer AL, Schatz JH, Zhao H, Staudt LM, and Singh H, “Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation,” Immunity, vol. 25, no. 2, pp. 225–236, 8. 2006, doi: 10.1016/j.immuni.2006.07.009. [PubMed: 16919487]

[18]. Willis SN et al. , “Transcription factor IRF4 regulates germinal center cell formation through a B cell-intrinsic mechanism,” J. Immunol. Baltim. Md 1950, vol. 192, no. 7, pp. 3200–3206, 4. 2014, doi: 10.4049/jimmunol.1303216.

[19]. Kwak K, Akkaya M, and Pierce SK, “B cell signaling in context,” Nat. Immunol, vol. 20, no. 8, pp. 963–969, 2019, doi: 10.1038/s41590-019-0427-9. [PubMed: 31285625]

[20]. Matsuyama T. et al. , “Molecular cloning of LSIRF, a lymphoid-specific member of the interferon regulatory factor family that binds the interferon-stimulated response element (ISRE),” Nucleic Acids Res., vol. 23, no. 12, pp. 2127–2136, 6. 1995, doi: 10.1093/nar/23.12.2127. [PubMed: 7541907]

[21]. Grumont RJ and Gerondakis S, “Rel induces interferon regulatory factor 4 (IRF-4) expression in lymphocytes: modulation of interferon-regulated gene expression by rel/nuclear factor kappaB,” J. Exp. Med, vol. 191, no. 8, pp. 1281–1292, 4. 2000, doi: 10.1084/jem.191.8.1281. [PubMed: 10770796]

[22]. Klein-Hessling S. et al. , “NFATc1 controls the cytotoxicity of CD8+ T cells,” Nat. Commun, vol. 8, no. 1, p. 511, 11 2017, doi: 10.1038/s41467-017-00612-6. [PubMed: 28894104]

[23]. Yao S. et al. , “Interferon regulatory factor 4 sustains CD8(+) T cell expansion and effector differentiation,” Immunity, vol. 39, no. 5, pp. 833–845, 11. 2013, doi: 10.1016/j.immuni.2013.10.007. [PubMed: 24211184]

[24]. Carrasco YR and Batista FD, “B cell recognition of membrane-bound antigen: an exquisite way of sensing ligands,” Curr. Opin. Immunol, vol. 18, no. 3, pp. 286–291, 6. 2006, doi: 10.1016/j.coi.2006.03.013. [PubMed: 16616474]

[25]. Davis MM, Krogsgaard M, Huse M, Huppa J, Lillemeier BF, and Li Q, “T cells as a self-referential, sensory organ,” Annu. Rev. Immunol, vol. 25, pp. 681–695, 2007, doi: 10.1146/annurev.immunol.24.021605.090600. [PubMed: 17291190]

[26]. Paus D, Phan TG, Chan TD, Gardam S, Basten A, and Brink R, “Antigen recognition strength regulates the choice between extrafollicular plasma cell and germinal center B cell differentiation,” J. Exp. Med, vol. 203, no. 4, pp. 1081–1091, 4. 2006, doi: 10.1084/jem.20060087. [PubMed: 16606676]

[27]. Shih T-AY, Meffre E, Roederer M, and Nussenzweig MC, “Role of BCR affinity in T cell dependent antibody responses in vivo,” Nat. Immunol, vol. 3, no. 6, pp. 570–575, 6. 2002, doi: 10.1038/ni803. [PubMed: 12021782]

[28]. Shih T-AY, Roederer M, and Nussenzweig MC, “Role of antigen receptor affinity in T cell-independent antibody responses in vivo,” Nat. Immunol, vol. 3, no. 4, pp. 399–406, 4. 2002, doi: 10.1038/ni776. [PubMed: 11896394]

[29]. Schwickert TA et al. , “A dynamic T cell-limited checkpoint regulates affinity-dependent B cell entry into the germinal center,” J. Exp. Med, vol. 208, no. 6, pp. 1243–1252, 6. 2011, doi: 10.1084/jem.20102477. [PubMed: 21576382]

[30]. Dal Porto JM, Haberman AM, Kelsoe G, and Shlomchik MJ, “Very low affinity B cells form germinal centers, become memory B cells, and participate in secondary immune responses when higher affinity competition is reduced,” J. Exp. Med, vol. 195, no. 9, pp. 1215–1221, 5 2002, doi: 10.1084/jem.20011550. [PubMed: 11994427]

[31]. Batista FD and Neuberger MS, “Affinity dependence of the B cell response to antigen: a threshold, a ceiling, and the importance of off-rate,” Immunity, vol. 8, no. 6, pp. 751–759, 6. 1998, doi: 10.1016/s1074-7613(00)80580-4. [PubMed: 9655489]

[32]. Kotov DI et al. , “TCR Affinity Biases Th Cell Differentiation by Regulating CD25, Eef1e1, and Gbp2,” J. Immunol. Baltim. Md 1950, vol. 202, no. 9, pp. 2535–2545, 01 2019, doi: 10.4049/jimmunol.1801609.

[33]. Keck S. et al. , “Antigen affinity and antigen dose exert distinct influences on CD4 T-cell differentiation,” Proc. Natl. Acad. Sci. U. S. A, vol. 111, no. 41, pp. 14852–14857, 10. 2014, doi: 10.1073/pnas.1403271111. [PubMed: 25267612]

[34]. Snook JP, Kim C, and Williams MA, “TCR signal strength controls the differentiation of CD4+ effector and memory T cells,” Sci. Immunol, vol. 3, no. 25, 20 2018, doi: 10.1126/sciimmunol.aas9103.

[35]. Taniguchi T, Ogasawara K, Takaoka A, and Tanaka N, “IRF family of transcription factors as regulators of host defense,” Annu. Rev. Immunol, vol. 19, pp. 623–655, 2001, doi: 10.1146/annurev.immunol.19.1.623. [PubMed: 11244049]

[36]. Takahasi K. et al. , “X-ray crystal structure of IRF-3 and its functional implications,” Nat. Struct. Biol, vol. 10, no. 11, pp. 922–927, 11. 2003, doi: 10.1038/nsb1001. [PubMed: 14555995]

[37]. Escalante CR, Yie J, Thanos D, and Aggarwal AK, “Structure of IRF-1 with bound DNA reveals determinants of interferon regulation,” Nature, vol. 391, no. 6662, pp. 103–106, 1. 1998, doi: 10.1038/34224. [PubMed: 9422515]

[38]. Mittrücker HW et al. , “Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function,” Science, vol. 275, no. 5299, pp. 540–543, 1. 1997, doi: 10.1126/science.275.5299.540. [PubMed: 8999800]

[39]. Klein U. et al. , “Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination,” Nat. Immunol, vol. 7, no. 7, pp. 773–782, 7. 2006, doi: 10.1038/ni1357. [PubMed: 16767092]

[40]. Sciammas R, Li Y, Warmflash A, Song Y, Dinner AR, and Singh H, “An incoherent regulatory network architecture that orchestrates B cell diversification in response to antigen signaling,” Mol. Syst. Biol, vol. 7, p. 495, 5 2011, doi: 10.1038/msb.2011.25. [PubMed: 21613984]

[41]. Eisenbeis CF, Singh H, and Storb U, “Pip, a novel IRF family member, is a lymphoidspecific, PU.1-dependent transcriptional activator,” Genes Dev., vol. 9, no. 11, pp. 1377–1387, 6. 1995, doi: 10.1101/gad.9.11.1377. [PubMed: 7797077]

[42]. Brass AL, Kehrli E, Eisenbeis CF, Storb U, and Singh H, “Pip, a lymphoid-restricted IRF, contains a regulatory domain that is important for autoinhibition and ternary complex formation with the Ets factor PU.1,” Genes Dev., vol. 10, no. 18, pp. 2335–2347, 9. 1996, doi: 10.1101/gad.10.18.2335. [PubMed: 8824592]

[43]. Glasmacher E. et al. , “A genomic regulatory element that directs assembly and function of immune-specific AP-1-IRF complexes,” Science, vol. 338, no. 6109, pp. 975–980, 11. 2012, doi: 10.1126/science.1228309. [PubMed: 22983707]

[44]. Tussiwand R. et al. , “Compensatory dendritic cell development mediated by BATF-IRF interactions,” Nature, vol. 490, no. 7421, pp. 502–507, 10. 2012, doi: 10.1038/nature11531. [PubMed: 22992524]

[45]. Li P. et al. , “BATF-JUN is critical for IRF4-mediated transcription in T cells,” Nature, vol. 490, no. 7421, pp. 543–546, 10. 2012, doi: 10.1038/nature11530. [PubMed: 22992523]

[46]. Ciofani M. et al. , “A validated regulatory network for Th17 cell specification,” Cell, vol. 151, no. 2, pp. 289–303, 10. 2012, doi: 10.1016/j.cell.2012.09.016. [PubMed: 23021777]

[47]. Iwata A. et al. , “Quality of TCR signaling determined by differential affinities of enhancers for the composite BATF-IRF4 transcription factor complex,” Nat. Immunol, vol. 18, no. 5, pp. 563–572, 2017, doi: 10.1038/ni.3714. [PubMed: 28346410]

[48]. Mancino A. et al. , “A dual cis-regulatory code links IRF8 to constitutive and inducible gene expression in macrophages,” Genes Dev., vol. 29, no. 4, pp. 394–408, 2. 2015, doi: 10.1101/gad.257592.114. [PubMed: 25637355]

[49]. Brass AL, Zhu AQ, and Singh H, “Assembly requirements of PU.1-Pip (IRF-4) activator complexes: inhibiting function in vivo using fused dimers,” EMBO J., vol. 18, no. 4, pp. 977–991, 2. 1999, doi: 10.1093/emboj/18.4.977. [PubMed: 10022840]

[50]. Nagulapalli S. and Atchison ML, “Transcription factor Pip can enhance DNA binding by E47, leading to transcriptional synergy involving multiple protein domains,” Mol. Cell. Biol, vol. 18, no. 8, pp. 4639–4650, 8. 1998, doi: 10.1128/mcb.18.8.4639. [PubMed: 9671474]

[51]. Ochiai K. et al. , “Zinc finger-IRF composite elements bound by Ikaros/IRF4 complexes function as gene repression in plasma cell,” Blood Adv., vol. 2, no. 8, pp. 883–894, 24 2018, doi: 10.1182/bloodadvances.2017010413. [PubMed: 29669755]

[52]. Rengarajan J, Mowen KA, McBride KD, Smith ED, Singh H, and Glimcher LH, “Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression,” J. Exp. Med, vol. 195, no. 8, pp. 1003–1012, 4. 2002, doi: 10.1084/jem.20011128. [PubMed: 11956291]

[53]. Kwon H. et al. , “Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors,” Immunity, vol. 31, no. 6, pp. 941–952, 12. 2009, doi: 10.1016/j.immuni.2009.10.008. [PubMed: 20064451]

[54]. Man K. et al. , “Transcription Factor IRF4 Promotes CD8+ T Cell Exhaustion and Limits the Development of Memory-like T Cells during Chronic Infection,” Immunity, vol. 47, no. 6, pp. 1129–1141.e5, 19 2017, doi: 10.1016/j.immuni.2017.11.021.

[55]. Mahnke J. et al. , “Interferon Regulatory Factor 4 controls TH1 cell effector function and metabolism,” Sci. Rep, vol. 6, p. 35521–20 2016, doi: 10.1038/srep35521. [PubMed: 27762344]

[56]. Vasanthakumar A. et al. , “The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells,” Nat. Immunol, vol. 16, no. 3, pp. 276–285, 3. 2015, doi: 10.1038/ni.3085. [PubMed: 25599561]

[57]. Matsumoto M. et al. , “Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation,” Immunity, vol. 41, no. 6, pp. 1040–1051, 12. 2014, doi: 10.1016/j.immuni.2014.10.016. [PubMed: 25484301]

[58]. Man K. et al. , “The transcription factor IRF4 is essential for TCR affinity-mediated metabolic programming and clonal expansion of T cells,” Nat. Immunol, vol. 14, no. 11, pp. 1155–1165, 11. 2013, doi: 10.1038/ni.2710. [PubMed: 24056747]

[59]. Nayar R. et al. , “Graded levels of IRF4 regulate CD8+ T cell differentiation and expansion, but not attrition, in response to acute virus infection,” J. Immunol. Baltim. Md 1950, vol. 192, no. 12, pp. 5881–5893, 6. 2014, doi: 10.4049/jimmunol.1303187.

[60]. Bollig N. et al. , “Transcription factor IRF4 determines germinal center formation through follicular T-helper cell differentiation,” Proc. Natl. Acad. Sci. U. S. A, vol. 109, no. 22, pp. 8664–8669, 5 2012, doi: 10.1073/pnas.1205834109. [PubMed: 22552227]

[61]. Cretney E. et al. , “The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells,” Nat. Immunol, vol. 12, no. 4, pp. 304–311, 4. 2011, doi: 10.1038/ni.2006. [PubMed: 21378976]

[62]. Biswas PS et al. , “Phosphorylation of IRF4 by ROCK2 regulates IL-17 and IL-21 production and the development of autoimmunity in mice,” J. Clin. Invest, vol. 120, no. 9, pp. 3280–3295, 9. 2010, doi: 10.1172/JCI42856. [PubMed: 20697158]

[63]. Staudt V. et al. , “Interferon-regulatory factor 4 is essential for the developmental program of T helper 9 cells,” Immunity, vol. 33, no. 2, pp. 192–202, 8. 2010, doi: 10.1016/j.immuni.2010.07.014. [PubMed: 20674401]

[64]. Ahyi A-NN, Chang H-C, Dent AL, Nutt SL, and Kaplan MH, "IFN regulatory factor 4 regulates the expression of a subset of Th2 cytokines," J. Immunol. Baltim. Md 1950, vol. 183, no. 3, pp. 1598–1606, 8. 2009, doi: 10.4049/jimmunol.0803302.

[65]. Zheng Y. et al. , "Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses," Nature, vol. 458, no. 7236, pp. 351–356, 3. 2009, doi: 10.1038/nature07674. [PubMed: 19182775]

[66]. Chen Q. et al. , "IRF-4-binding protein inhibits interleukin-17 and interleukin-21 production by controlling the activity of IRF-4 transcription factor," Immunity, vol. 29, no. 6, pp. 899–911, 12. 2008, doi: 10.1016/j.immuni.2008.10.011. [PubMed: 19062315]

[67]. Mudter J. et al. , "The transcription factor IFN regulatory factor-4 controls experimental colitis in mice via T cell-derived IL-6," J. Clin. Invest, vol. 118, no. 7, pp. 2415–2426, 7. 2008, doi: 10.1172/JCI33227. [PubMed: 18535667]

[68]. Brüstle A. et al. , "The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4," Nat. Immunol, vol. 8, no. 9, pp. 958–966, 9. 2007, doi: 10.1038/ni1500. [PubMed: 17676043]

[69]. Tominaga N, Ohkusu-Tsukada K, Udono H, Abe R, Matsuyama T, and Yui K, "Development of Th1 and not Th2 immune responses in mice lacking IFN-regulatory factor-4," Int. Immunol, vol. 15, no. 1, pp. 1–10, 1. 2003, doi: 10.1093/intimm/dxg001. [PubMed: 12502720]

[70]. Lohoff M. et al. , "Dysregulated T helper cell differentiation in the absence of interferon regulatory factor 4," Proc. Natl. Acad. Sci. U. S. A, vol. 99, no. 18, pp. 11808–11812, 9. 2002, doi: 10.1073/pnas.182425099. [PubMed: 12189207]

[71]. Gupta S, Jiang M, Anthony A, and Pernis AB, "Lineage-specific modulation of interleukin 4 signaling by interferon regulatory factor 4," J. Exp. Med, vol. 190, no. 12, pp. 1837–1848, 12. 1999, doi: 10.1084/jem.190.12.1837. [PubMed: 10601358]

[72]. Ye BH, Rao PH, Chaganti RS, and Dalla-Favera R, "Cloning of bcl-6, the locus involved in chromosome translocations affecting band 3q27 in B-cell lymphoma," Cancer Res., vol. 53, no. 12, pp. 2732–2735, 6. 1993. [PubMed: 8504412]

[73]. Albagli O. et al. , "Overexpressed BCL6 (LAZ3) oncoprotein triggers apoptosis, delays S phase progression and associates with replication foci," Oncogene, vol. 18, no. 36, pp. 5063–5075, 9. 1999, doi: 10.1038/sj.onc.1202892. [PubMed: 10490843]

[74]. Kumagai T. et al. , "The proto-oncogene Bc16 inhibits apoptotic cell death in differentiation-induced mouse myogenic cells," Oncogene, vol. 18, no. 2, pp. 467–475, 1. 1999, doi: 10.1038/sj.onc.1202306. [PubMed: 9927203]

[75]. Yamochi T, Kaneita Y, Akiyama T, Mori S, and Moriyama M, "Adenovirus-mediated high expression of BCL-6 in CV-1 cells induces apoptotic cell death accompanied by downregulation of BCL-2 and BCL-X(L)," Oncogene, vol. 18, no. 2, pp. 487–494, 1. 1999, doi: 10.1038/sj.onc.1202334. [PubMed: 9927205]

[76]. Huynh KD and Bardwell VJ, "The BCL-6 POZ domain and other POZ domains interact with the co-repressors N-CoR and SMRT," Oncogene, vol. 17, no. 19, pp. 2473–2484, 11. 1998, doi: 10.1038/sj.onc.1202197. [PubMed: 9824158]

[77]. Dhordain P. et al. , "The LAZ3(BCL-6) oncoprotein recruits a SMRT/mSIN3A/histone deacetylase containing complex to mediate transcriptional repression," Nucleic Acids Res., vol. 26, no. 20, pp. 4645–4651, 10. 1998, doi: 10.1093/nar/26.20.4645. [PubMed: 9753732]

[78]. Seyfert VL, Allman D, He Y, and Staudt LM, "Transcriptional repression by the proto-oncogene BCL-6," Oncogene, vol. 12, no. 11, pp. 2331–2342, 6. 1996. [PubMed: 8649773]

[79]. Dent AL, Shaffer AL, Yu X, Allman D, and Staudt LM, "Control of inflammation, cytokine expression, and germinal center formation by BCL-6," Science, vol. 276, no. 5312, pp. 589–592, 4. 1997, doi: 10.1126/science.276.5312.589. [PubMed: 9110977]

[80]. Ye BH et al. , "The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation," Nat. Genet, vol. 16, no. 2, pp. 161–170, 6. 1997, doi: 10.1038/ng0697-161. [PubMed: 9171827]

[81]. Cattoretti G. et al. , "Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice," Cancer Cell, vol. 7, no. 5, pp. 445–455, 5 2005, doi: 10.1016/j.ccr.2005.03.037. [PubMed: 15894265]

[82]. Shaffer AL, Yu X, He Y, Boldrick J, Chan EP, and Staudt LM, "BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control," Immunity, vol. 13, no. 2, pp. 199–212, 8. 2000, doi: 10.1016/s1074-7613(00)00020-0. [PubMed: 10981963]

[83]. Shaffer AL et al. , "Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program," Immunity, vol. 17, no. 1, pp. 51–62, 7. 2002, doi: 10.1016/s1074-7613(02)00335-7. [PubMed: 12150891]

[84]. Sciammas R. and Davis MM, "Modular nature of Blimp-1 in the regulation of gene expression during B cell maturation," J. Immunol. Baltim. Md 1950, vol. 172, no. 9, pp. 5427–5440, 5 2004, doi: 10.4049/jimmunol.172.9.5427.

[85]. Minnich M. et al. , "Multifunctional role of the transcription factor Blimp-1 in coordinating plasma cell differentiation," Nat. Immunol, vol. 17, no. 3, pp. 331–343, 3. 2016, doi: 10.1038/ni.3349. [PubMed: 26779602]

[86]. Turner CA, Mack DH, and Davis MM, "Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells," Cell, vol. 77, no. 2, pp. 297–306, 4. 1994, doi: 10.1016/0092-8674(94)90321-2. [PubMed: 8168136]

[87]. Lin K-I, Angelin-Duclos C, Kuo TC, and Calame K, "Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells," Mol. Cell. Biol, vol. 22, no. 13, pp. 4771–4780, 7. 2002, doi: 10.1128/mcb.22.13.4771-4780.2002. [PubMed: 12052884]

[88]. Shapiro-Shelef M, Lin K-I, McHeyzer-Williams LJ, Liao J, McHeyzer-Williams MG, and Calame K, "Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells.," Immunity, vol. 19, no. 4, pp. 607–620, 10. 2003, doi: 10.1016/s1074-7613(03)00267-x. [PubMed: 14563324]

[89]. Falini B. et al. , "A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells," Blood, vol. 95, no. 6, pp. 2084–2092, 3. 2000. [PubMed: 10706878]

[90]. Teng Y. et al. , "IRF4 negatively regulates proliferation of germinal center B cell-derived Burkitt's lymphoma cell lines and induces differentiation toward plasma cells," Eur. J. Cell Biol, vol. 86, no. 10, pp. 581–589, 10. 2007, doi: 10.1016/j.ejcb.2007.05.006. [PubMed: 17651861]

[91]. Saito M. et al. , "A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma," Cancer Cell, vol. 12, no. 3, pp. 280–292, 9. 2007, doi: 10.1016/j.ccr.2007.08.011. [PubMed: 17785208]

[92]. Choi YS et al. , "ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6," Immunity, vol. 34, no. 6, pp. 932–946, 6. 2011, doi: 10.1016/j.immuni.2011.03.023. [PubMed: 21636296]

[93]. Fazilleau N, McHeyzer-Williams LJ, Rosen H, and McHeyzer-Williams MG, "The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding," Nat. Immunol, vol. 10, no. 4, pp. 375–384, 4. 2009, doi: 10.1038/ni.1704. [PubMed: 19252493]

[94]. Tubo NJ et al. , "Single naive CD4+ T cells from a diverse repertoire produce different effector cell types during infection," Cell, vol. 153, no. 4, pp. 785–796, 5 2013, doi: 10.1016/j.cell.2013.04.007. [PubMed: 23663778]

[95]. Roco JA et al. , "Class-Switch Recombination Occurs Infrequently in Germinal Centers," Immunity, vol. 51, no. 2, pp. 337–350.e7, 20 2019, doi: 10.1016/j.immuni.2019.07.001. [PubMed: 31375460]

[96]. Ci W. et al. , "The BCL6 transcriptional program features repression of multiple oncogenes in primary B cells and is deregulated in DLBCL," Blood, vol. 113, no. 22, pp. 5536–5548, 5 2009, doi: 10.1182/blood-2008-12-193037. [PubMed: 19307668]

[97]. Li X. et al. , "Cbl Ubiquitin Ligases Control B Cell Exit from the Germinal-Center Reaction," Immunity, vol. 48, no. 3, pp. 530–541.e6, 20 2018, doi: 10.1016/j.immuni.2018.03.006. [PubMed: 29562201]

[98]. Pancer Z, Mayer WE, Klein J, and Cooper MD, "Prototypic T cell receptor and CD4-like coreceptor are expressed by lymphocytes in the agnathan sea lamprey," Proc. Natl. Acad. Sci. U. S. A, vol. 101, no. 36, pp. 13273–13278, 9. 2004, doi: 10.1073/pnas.0405529101. [PubMed: 15328402]

[99]. Suzuki T, Shin-I T, Fujiyama A, Kohara Y, and Kasahara M, "Hagfish leukocytes express a paired receptor family with a variable domain resembling those of antigen receptors," J. Immunol. Baltim. Md 1950, vol. 174, no. 5, pp. 2885–2891, 3. 2005, doi: 10.4049/jimmunol.174.5.2885.

[100]. Yu C. et al. , "Genes 'waiting' for recruitment by the adaptive immune system: the insights from amphioxus," J. Immunol. Baltim. Md 1950, vol. 174, no. 6, pp. 3493–3500, 3. 2005, doi: 10.4049/jimmunol.174.6.3493.

[101]. Fernando TM et al. , "BCL6 Evolved to Enable Stress Tolerance in Vertebrates and Is Broadly Required by Cancer Cells to Adapt to Stress," Cancer Discov., vol. 9, no. 5, pp. 662–679, 2019, doi: 10.1158/2159-8290.CD-17-1444. [PubMed: 30777872]

[102]. Castro CD, Ohta Y, Dooley H, and Flajnik MF, "Noncoordinate expression of J-chain and Blimp-1 define nurse shark plasma cell populations during ontogeny," Eur. J. Immunol, vol. 43, no. 11, pp. 3061–3075, 11. 2013, doi: 10.1002/eji.201343416. [PubMed: 23897025]

[103]. Livi CB and Davidson EH, "Expression and function of blimp1/krox, an alternatively transcribed regulatory gene of the sea urchin endomesoderm network," Dev. Biol, vol. 293, no. 2, pp. 513–525, 5 2006, doi: 10.1016/j.ydbio.2006.02.021. [PubMed: 16581059]

[104]. Du K. et al. , "Ancient duplications and functional divergence in the interferon regulatory factors of vertebrates provide insights into the evolution of vertebrate immune systems," Dev. Comp. Immunol, vol. 81, pp. 324–333, 2018, doi: 10.1016/j.dci.2017.12.016. [PubMed: 29253557]

[105]. Arendt D. et al. , "The origin and evolution of cell types," Nat. Rev. Genet, vol. 17, no. 12, pp. 744–757, 2016, doi: 10.1038/nrg.2016.127. [PubMed: 27818507]

[106]. Thompson CB, "New insights into V(D)J recombination and its role in the evolution of the immune system," Immunity, vol. 3, no. 5, pp. 531–539, 11. 1995, doi: 10.1016/1074-7613(95)90124-8. [PubMed: 7584143]

[107]. Klein J. and Nikolaidis N, "The descent of the antibody-based immune system by gradual evolution," Proc. Natl. Acad. Sci. U. S. A, vol. 102, no. 1, pp. 169–174, 1. 2005, doi: 10.1073/pnas.0408480102. [PubMed: 15618397]

[108]. Kasahara M, Suzuki T, and Pasquier LD, "On the origins of the adaptive immune system: novel insights from invertebrates and cold-blooded vertebrates," Trends Immunol., vol. 25, no. 2, pp. 105–111, 2. 2004, doi: 10.1016/j.it.2003.11.005. [PubMed: 15102370]

[109]. Hart Y. and Alon U, "The utility of paradoxical components in biological circuits," Mol. Cell, vol. 49, no. 2, pp. 213–221, 1. 2013, doi: 10.1016/j.molcel.2013.01.004. [PubMed: 23352242]

[110]. Shaffer AL et al. , "A library of gene expression signatures to illuminate normal and pathological lymphoid biology," Immunol. Rev, vol. 210, pp. 67–85, 4. 2006, doi: 10.1111/j.0105-2896.2006.00373.x. [PubMed: 16623765]

[111]. Victora GD et al. , "Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter," Cell, vol. 143, no. 4, pp. 592–605, 11. 2010, doi: 10.1016/j.cell.2010.10.032. [PubMed: 21074050]

[112]. Ise W. et al. , "T Follicular Helper Cell-Germinal Center B Cell Interaction Strength Regulates Entry into Plasma Cell or Recycling Germinal Center Cell Fate," Immunity, vol. 48, no. 4, pp. 702–715.e4, 17 2018, doi: 10.1016/j.immuni.2018.03.027. [PubMed: 29669250]

[113]. Guérin A. et al. , "IRF4 haploinsufficiency in a family with Whipple's disease," eLife, vol. 7, 14 2018, doi: 10.7554/eLife.32340.

[114]. Rutishauser RL et al. , "Transcriptional repressor Blimp-1 promotes CD8(+) T cell terminal differentiation and represses the acquisition of central memory T cell properties," Immunity, vol. 31, no. 2, pp. 296–308, 8. 2009, doi: 10.1016/j.immuni.2009.05.014. [PubMed: 19664941]

[115]. Vander Lugt B. et al. , "Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation," Nat. Immunol, vol. 15, no. 2, pp. 161–167, 2. 2014, doi: 10.1038/ni.2795. [PubMed: 24362890]

[116]. Williams JW et al. , "Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation," Nat. Commun, vol. 4, p. 2990–2013, doi: 10.1038/ncomms3990.

[117]. Huang SC-C et al. , “Metabolic Reprogramming Mediated by the mTORC2-IRF4 Signaling Axis Is Essential for Macrophage Alternative Activation,” Immunity, vol. 45, no. 4, pp. 817–830, 18 2016, doi: 10.1016/j.immuni.2016.09.016. [PubMed: 27760338]

[118]. Gao Y. et al. , “Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells,” Immunity, vol. 39, no. 4, pp. 722–732, 10. 2013, doi: 10.1016/j.immuni.2013.08.028. [PubMed: 24076050]

[119]. Zhang T, Liu D, Calabro S, Eisenbarth SC, Cattoretti G, and Haberman AM, “Dynamic expression of BCL6 in murine conventional dendritic cells during in vivo development and activation,” PloS One, vol. 9, no. 6, p. e101208, 2014, doi: 10.1371/journal.pone.0101208.

[120]. Kim SJ, Zou YR, Goldstein J, Reizis B, and Diamond B, “Tolerogenic function of Blimp-1 in dendritic cells,” J. Exp. Med, vol. 208, no. 11, pp. 2193–2199, 10. 2011, doi: 10.1084/jem.20110658. [PubMed: 21948081]

[121]. Stone SL et al. , “T-bet Transcription Factor Promotes Antibody-Secreting Cell Differentiation by Limiting the Inflammatory Effects of IFN-γ on B Cells,” Immunity, vol. 50, no. 5, pp. 1172–1187.e7, 21 2019, doi: 10.1016/j.immuni.2019.04.004.

[122]. Manni M. et al. , “Regulation of age-associated B cells by IRF5 in systemic autoimmunity,” Nat. Immunol, vol. 19, no. 4, pp. 407–419, 2018, doi: 10.1038/s41590-018-0056-8. [PubMed: 29483597]

[123]. Roy K. et al. , “A Regulatory Circuit Controlling the Dynamics of NFκB cRel Transitions B Cells from Proliferation to Plasma Cell Differentiation,” Immunity, vol. 50, no. 3, pp. 616–628.e6, 19 2019, doi: 10.1016/j.immuni.2019.02.004. [PubMed: 30850343]

[124]. Gottschalk RA, Martins AJ, Sjoelund V, Angermann BR, Lin B, and Germain RN, “Recent progress using systems biology approaches to better understand molecular mechanisms of immunity,” Semin. Immunol, vol. 25, no. 3, pp. 201–208, 10. 2013, doi: 10.1016/j.smim.2012.11.002. [PubMed: 23238271]

[125]. Duffy KR et al. , “Activation-induced B cell fates are selected by intracellular stochastic competition,” Science, vol. 335, no. 6066, pp. 338–341, 1. 2012, doi: 10.1126/science.1213230. [PubMed: 22223740]

[126]. Urban EA and Johnston RJ, “Buffering and Amplifying Transcriptional Noise During Cell Fate Specification,” Front. Genet, vol. 9, p. 591, 2018, doi: 10.3389/fgene.2018.00591. [PubMed: 30555516]

[127]. Tarlinton DM, “Evolution in miniature: selection, survival and distribution of antigen reactive cells in the germinal centre,” Immunol. Cell Biol, vol. 86, no. 2, pp. 133–138, 2. 2008, doi: 10.1038/sj.icb.7100148. [PubMed: 18180800]

[128]. Barwick BG, Scharer CD, Bally APR, and Boss JM, “Plasma cell differentiation is coupled to division-dependent DNA hypomethylation and gene regulation,” Nat. Immunol, vol. 17, no. 10, pp. 1216–1225, 2016, doi: 10.1038/ni.3519. [PubMed: 27500631]

[129]. Cho SH et al. , “Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system,” Nature, vol. 537, no. 7619, pp. 234–238, 08 2016, doi: 10.1038/nature19334. [PubMed: 27501247]

[130]. Chou C. et al. , “The Transcription Factor AP4 Mediates Resolution of Chronic Viral Infection through Amplification of Germinal Center B Cell Responses,” Immunity, vol. 45, no. 3, pp. 570–582, 20 2016, doi: 10.1016/j.immuni.2016.07.023. [PubMed: 27566940]

[131]. Lam WY et al. , “Mitochondrial Pyruvate Import Promotes Long-Term Survival of Antibody-Secreting Plasma Cells,” Immunity, vol. 45, no. 1, pp. 60–73, 19 2016, doi: 10.1016/j.immuni.2016.06.011. [PubMed: 27396958]

[132]. Ray JP et al. , “The Interleukin-2-mTORc1 Kinase Axis Defines the Signaling, Differentiation, and Metabolism of T Helper 1 and Follicular B Helper T Cells,” Immunity, vol. 43, no. 4, pp. 690–702, 10. 2015, doi: 10.1016/j.immuni.2015.08.017. [PubMed: 26410627]

[133]. Johnson MO et al. , “Distinct Regulation of Th17 and Th1 Cell Differentiation by Glutaminase-Dependent Metabolism,” Cell, vol. 175, no. 7, pp. 1780–1795.e19, 13 2018, doi: 10.1016/j.cell.2018.10.001.

[134]. Shoval O. and Alon U, “SnapShot: network motifs,” Cell, vol. 143, no. 2, pp. 326–e1, 10. 2010, doi: 10.1016/j.cell.2010.09.050. [PubMed: 20946989]

Box 1

State-specific gene programs and Gene Regulatory Networks (GRNs)

Upon antigen recognition, B and T cells integrate the second messenger signals of cell activation to drive cellular differentiation. Many key TFs and epigenetic mechanisms, as well as miRNAs and lncRNAs, have been identified that orchestrate necessary changes in gene expression [1]. Intense research over the past 15 years has illuminated the key role of GRNs in specification and commitment of diverse effector cell fates [109]. GRNs are assemblies of both positively and negatively acting TFs that operate to drive gene programs important for specific cell fate trajectories. As such, distinct GRNs are deployed that depend on a unique set of transcriptional regulators for each cell fate. Because a given naïve cell and its corresponding antigen receptor is thought to be capable of differentiating into any given effector cell, it is hypothesized that cell state-specific GRNs are coordinated with each other in a dynamic manner to allocate the correct proportion of each cell type appropriate for a given immune response [124]. The dynamics of a GRN are governed by both positive and negative inputs as well as by biophysical features that include time, abundance and affinity. Thus, the architecture of a GRN is key to the precise and timely allocation of cell fate [134]. Coherent networks function in a feed-forward manner to commit to a specified cell fate. Conversely, incoherent or paradoxical networks enact both positive and negative regulation on the same target that enable temporal or fold change detection capabilities in response to graded inputs [109]. For example, signal strength of the antigen receptor's response to antigen is a graded input. This review focuses on a conserved, incoherent GRN, linked to the antigen receptor of both B and $CD4^+$ T cells to shape the antibody response by cell type allocation. Specifically, the incoherent GRN is based on a single activator, IRF4, which regulates the expression of two counter-antagonistic cell fate determinants, Bcl6 and Blimp-1.

Box 2.

Modeling the IRF4-based Gene Regulatory Network (GRN)

The IRF4 GRN is an incoherent network, where a single activator (IRF4) functions to promote the expression of two mutually antagonistic cell fate determinants (Bcl6/Blimp-1). A multi-scale mathematical simulation has been used to elucidate these dynamics over time [40]. Ordinary differential equations estimated the synthesis and degradation rates of multiple molecular species into the strength of a given regulatory interaction and were applied to the GRN as a function of varying IRF4 amounts. When the choice to undergo CSR or PC differentiation was analyzed, a dynamic emerged termed “kinetic control”. The kinetic control model states that activated B cells pass through an obligate state that promotes CSR prior to becoming a PC[40]. This obligate state is unstable when IRF4 accumulates to high amounts and antibody diversification is aborted in favor of antibody secretion. In contrast to a bifurcating cell fate decision, where B cells either diversify their antibody or secrete their antibody, kinetic control suggests that all cells gain the ability to diversify but this opportunity is terminated if a given cell accumulates high amounts of IRF4[40]. Kinetic control also likely coordinates the timing of CSR *in vivo* [95]. Like CSR, the GCB state also involves antibody diversification and exhibits low amounts of IRF4 expression; however, GCBs are stable unless the BCR and CD40 are appropriately engaged during selection. To accommodate the new findings of IRF4’s role in GCBs [16], [18], the original kinetic control model would need an additional negatively regulatory node to prevent further IRF4 accumulation and to maintain the GCB state. Potential negative regulatory candidates include Bcl6, which binds the IRF4 locus [91], [96], and Cbl-b and c-Cbl, which degrade IRF4 protein [97].

Given the conservation of the IRF4 GRN in B and $CD4^+$ cells, we speculate that the kinetic control model might also function in Tfh –Teff cell fate choices, where the Tfh state represents the obligate state through which all $CD4^+$ T cells pass. However, despite the similarities, the maintenance of IRF4 expression in differentiated $CD4^+$ T and B cells is unique to each lineage. Specifically, PCs require and maintain high amounts of IRF4. In contrast, in protein-adjuvant immunization settings, both Teff and Tfh cells express comparable IRF4 amounts [15], suggesting that $CD4^+$ T cells might retain memory of antigen encounters by adopting different fates, but not by the amount of IRF4 they express. As Blimp-1 is often associated with irreversible terminal differentiation, dampening IRF4 expression in Teff cells might be one of many mechanisms that maintain plasticity of $CD4^+$ T cell responses. A fuller interpretation of these results awaits analysis of IRF4 expression dynamics in infectious systems where antigen availability is actively replenished.

Box 3.

A Conserved IRF4 Gene Regulatory Network in the immune system?

The striking conservation of the IRF4 GRN in both B and $CD4^+$ T cells suggests that the GRN may have preceded the evolutionary bifurcation of T and B cells.In line with this, the family of innate immune receptors “Agnathan paired receptors resembling antigen receptors” (APAR) may have served as the ancestral vertebrate antigen receptor that also possessed signaling capacity [98]–[100]. Given the signaling propensity of the APAR, we speculate that the IRF4 GRN may have been fixed at that point of evolutionary time. In fact, IRF4, Bcl6, and Blimp-1 homologs have been identified in the ancient genomes of sea urchins and agnathans [101]–[104]. Given the multiple interfaces for interaction (e.g. protein-protein, protein-DNA elements) orchestrated to execute function, it is plausible that the IRF4 GRN arose from an ancestral apomere (cell type-specific cellular module) [105] already present in agnathans and has been evolutionarily constrained. As such, the IRF4 GRN could have been inherited during the emergence of adaptive immunity and the diversification of the variable component of the ancestral receptor in gnathostomes [106]–[108]. Although it is unclear what linked the IRF4 activator to Blimp-1 and Bcl6 fate determinants, a common theme in development is the pairing of counter-antagonistic regulators to enable alternate cell fates [109]. In contrast to all other members of the IRF family, IRF4 is not induced by either Type I or II IFN signaling [20]; instead, its regulation is largely governed by NFkB signaling [21], [71], [91], [110]. However, the nature of the selective forces enabling this shift during the course of apomere inheritance from agnathans is speculative. Despite this divergence, IRF4 has maintained the wide-ranging roles of IRFs in general cell biology, as evidenced by that of IRF4 in lymphocyte activation, metabolism, and differentiation. Notably, IRF4 loss-of-function variants are extremely rare in most human genome-wide association studies, leading to the hypothesis that IRF4 is subject to purifying selection, consistent with its essential role in immunity [113]. This finding makes it unlikely that the IRF4 GRN is only deployed for the initiation of naïve B and $CD4^+$ T cell responses. GCBs undergoing cell selection during affinity maturation “test” their SHM-mutated antibody, resulting in BCR and CD40 signaling, which resembles the initial steps of Tdependent B cell responses [29], [111], [112]. The IRF4 GRN also plays a role in $CD8^+$ T cytolytic responses, where IRF4 expression scales with TCR signal strength and IRF4 gene dosage affects the proportions and downstream cell states of $CD8^+$ T cells [114] [58]. Further, IRF4 is expressed in monocytes and dendritic cells where it also plays an essential role in cell fate decisions [115]–[118]; however, we note that it is unclear whether those decisions are dependent on Blimp-1 or Bcl6 [116], [119], [120].

Outstanding Questions

- Does the IRF4 GRN play a role in GCBs during affinity maturation? Since BCR and CD40 signals play a role in selection of affinity matured variants and BCR and CD40 signals regulate IRF4 amounts, this raises the possibility that the IRF4-dependent GRN is used in affinity maturation.
- Where is the bifurcation of memory B and $CD4^+$ T cells in the progenitor (naïve) to progeny (effector) map of the antibody response? Given that cell fate trajectory is responsive to BCR signaling and $CD4^+$ T cell help, this raises the possibility that IRF4 abundance plays a role in influencing the antibody response.
- How is the IRF4 GRN layered in the epigenetic and nuclear landscape of transitioning cells? As cells transition from progenitors to differentiated cell states, they undergo extensive epigenetic changes that may intersect with the IRF4 GRN.
- Does IRF4 control the metabolic changes associated with B and $CD4^+$ Tcell fate transitions and do the metabolic products feedback to shape cell fate? Metabolic products have emerged as critical mediators of cell state dynamics, including epigenetics, indicating that metabolic re-programming may influence the outcome of the IRF4-dependent GRN.
- Do post-translational mechanisms play a role in the dynamics of the IRF4 GRN? Post-translational regulation is a relatively understudied, yet important, feature of cell state.
- As IRF4 appears to be a central node controlling cell state and differentiation in these immune cell subsets, could future therapies targeting IRF4 improve specific disease outcomes? IRF4 has already been implicated in modulating allograft and cancer survival for certain cases, suggesting that manipulation of the IRF4 GRN might be beneficial for specific diseases, pending robust investigation.

Highlights

- The antibody response is orchestrated by the development of distinct B and $CD4^+$ T cell fates with specialized function.
- Distinct B and $CD4^+$ T cell fates are controlled by an antigen receptor signal strength-responsive, incoherent gene regulatory network, determined in mice
- The transcription factor IRF4 is a central and essential node of this network, whose expression scales with the intensity of antigen receptor signal strength
- Graded expression and differential DNA affinity of IRF4 can function to control the outcome of a counter-antagonistic gene regulatory interactions governing alternate cell fates

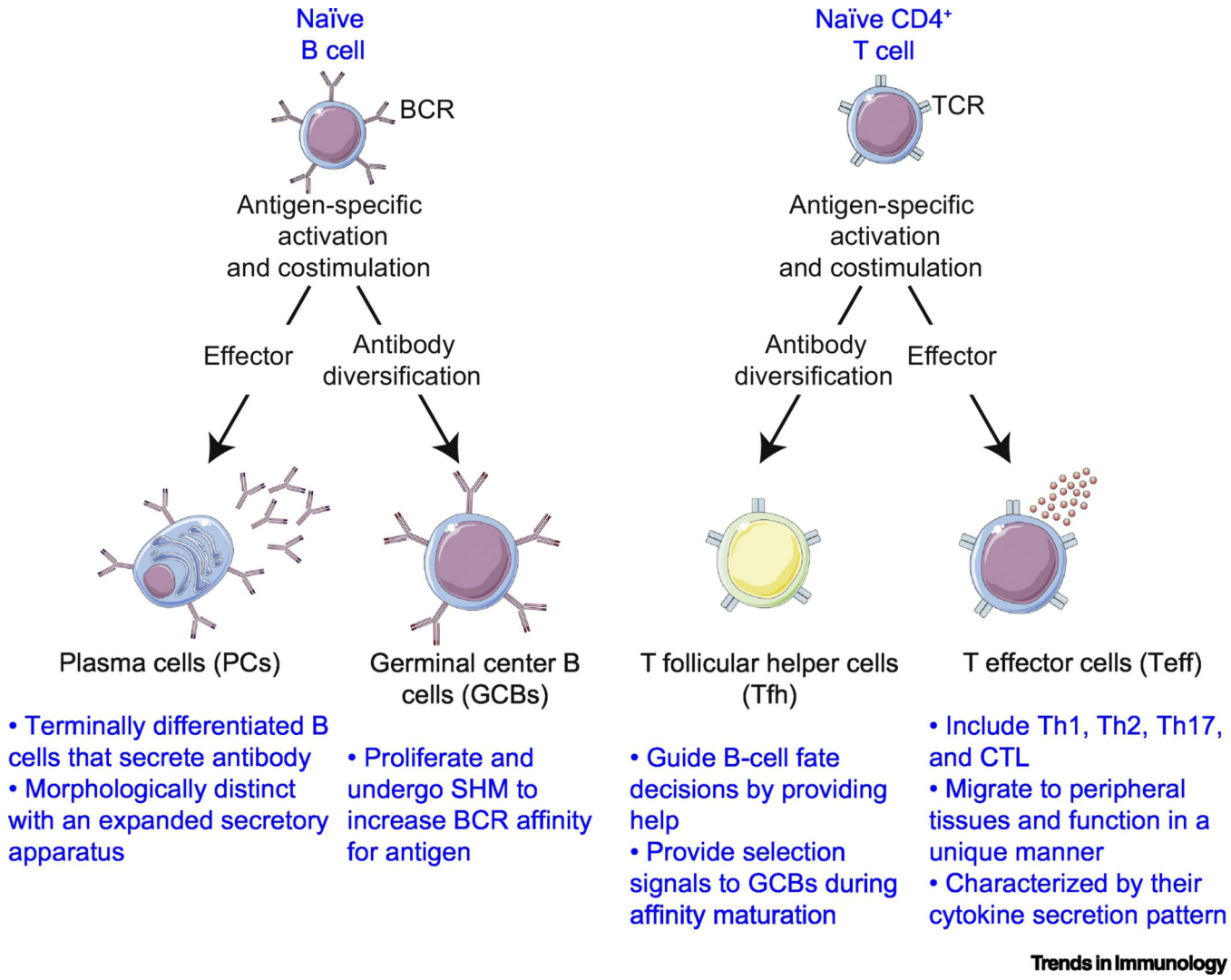


Figure 1. Naïve B and CD4$^+$ T cell progenitors activate and differentiate into progeny that can act as either effectors, or cells inducing antibody diversification in vertebrates.

Upon antigen specific activation and co-stimulation, B cells can terminally differentiate into effector, antibody secreting plasma cells (PC). Conversely, the B cell can participate in the germinal center and undergo affinity maturation - a process of mutation and selection that modifies the antigen specific BCR repertoire. Likewise, after antigen specific activation and co-stimulation, CD4$^+$T cells can terminally differentiate into effector (Teff), pro-inflammatory cytokine secreting cells. Alternatively, the CD4$^+$ T cell differentiates into a T follicular helper cell (Tfh), and participates in the germinal center reaction to select high affinity B cells (germinal center B cells: GCB). Not discussed here are progenitor memory cells - long lived cells for rapid reactivation upon a second antigen encounter. However, where memory cells arise in this process remains to be determined.

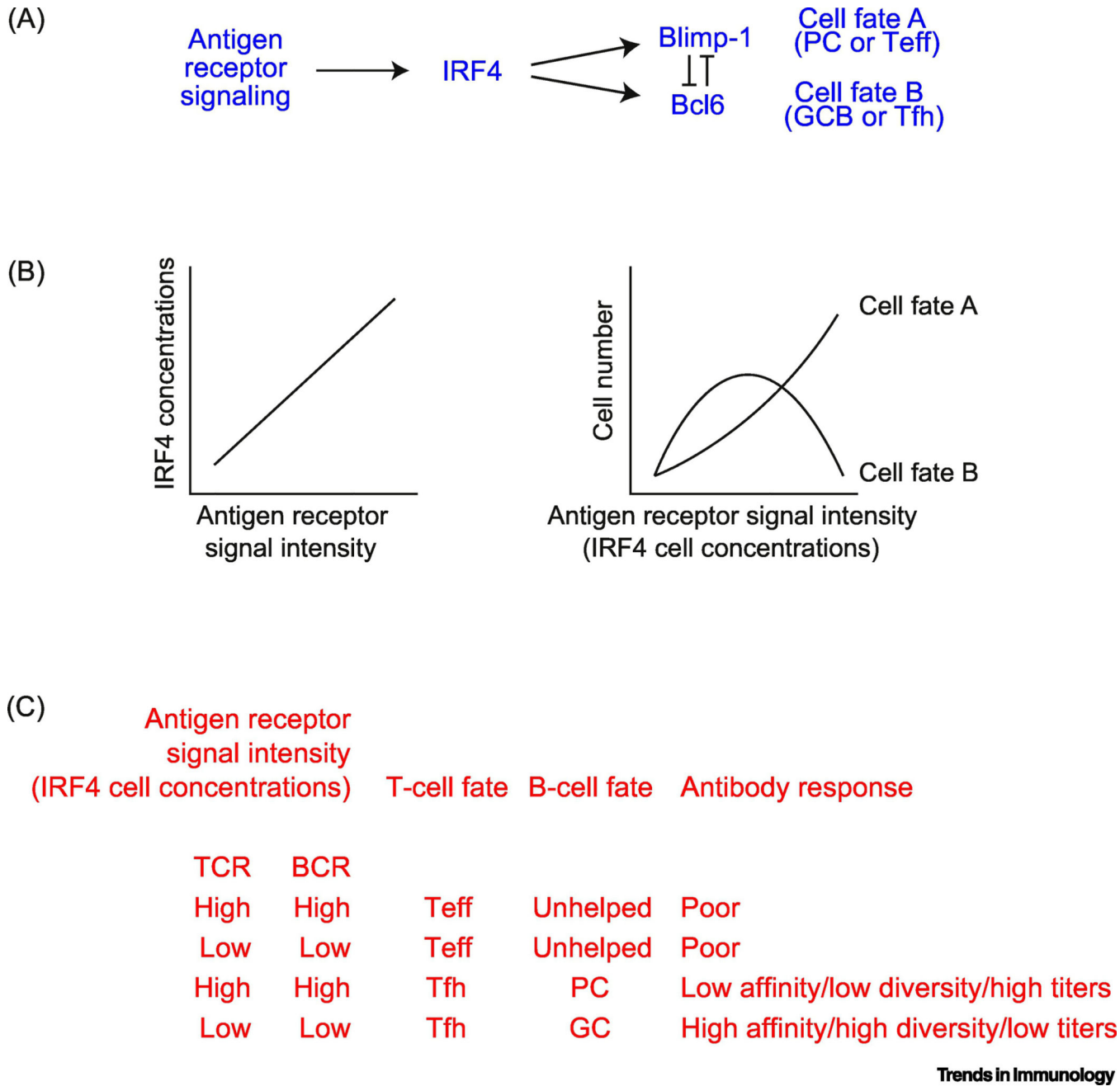
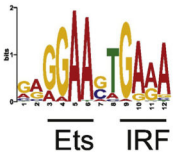
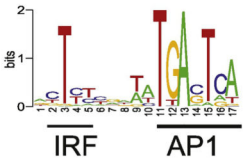
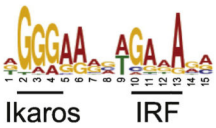
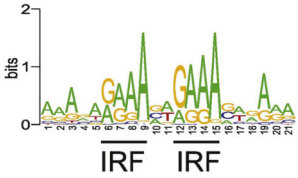


Figure 2. (Key Figure). Variations in the antibody response by independent control of IRF4 concentrations in B and CD4$^+$ T cells.

A) Topology of the IRF4-dependent GRN orchestrating alternative cell fate choice. The network combines an activating connection between the antigen receptor and IRF4 with an incoherent type 1 feedforward edge topology (from IRF4 to Blimp-1/Bcl6). B) Left, Intensity of antigen receptor signaling scales with IRF4 concentrations. Right, dynamics of the type 1 incoherent feedforward motif in regulating alternate cell fate choice as a function of antigen receptor signal intensity / IRF4 concentrations. The architecture of the incoherent motif is predicted to compute fold change detection of IRF4 concentrations to execute alternate cell fate choice by regulation of Blimp-1 or Bcl6. C) During T-dependent antibody

responses, independent control of IRF4 concentrations by antigen receptor signal intensity in T and B cells is predicted to result in different outcomes on the quantity and quality of the antibody response. PC: plasma cells; GCB: germinal center B cells; Teff: effector T cell; Tfh: T follicular helper cell.

DNA recognition site	Binding Partner	Relative DNA affinity[a]	Aligned with Gene Program	Sequence Logo
EICE Ets-IRF Composite Element	PU.1 or SpiB	high	B cell GCB	
AICE[b] AP1-IRF Composite Element	BATF/JunB or BATF/c-jun	high	B cell GCB/ T cell Tfh	
ZICE Ikaros-IRF Composite Element	Ikaros	high	B cell PC	
ISRE Interferon-stimulated Response Element	IRF4 (homodimer)[c]	low	B cell PC/ T cell Teff	

Trends in Immunology

Figure 3. Antigen dependent alternate B cell fate decisions.

Antigen recognition induces the expression of IRF4 in naïve B cells. Intensity of BCR and CD40 signaling scale with induced IRF4 concentrations. Higher IRF4 amounts favors the induction of Blimp-1 expression and preferential PC cell fates. Alternatively, lower IRF4 amounts favor the induction of Bcl6 expression and preferential germinal center B cells (GCB) cell fates. Varying IRF4 concentrations affect its partitioning into distinct assemblies of DNA binding partners and DNA recognition elements that are aligned with components of distinct gene programs to effect cell fate choice, including that of Blimp-1 and Bcl6[16]. PC: plasma cells.

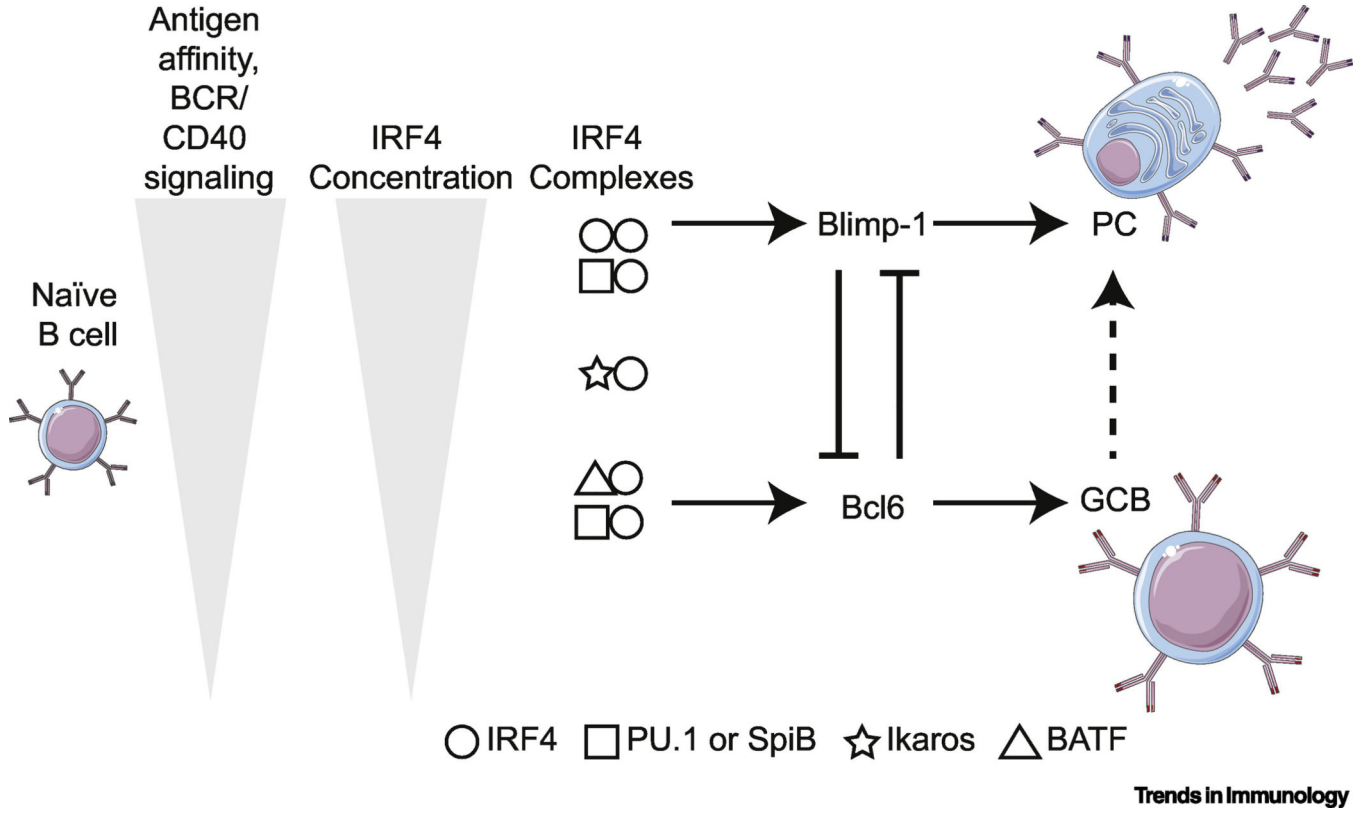


Figure 4. Antigen dependent alternate CD4^{+} T cell fate decisions.

Antigen recognition induces the expression of IRF4. Intensity of TCR signaling and costimulation scale with induced IRF4 concentrations. Higher IRF4 amounts favors the induction of Blimp-1 expression and preferential effector T cell (Teff) cell fates. Alternatively, lower IRF4 amounts favor the induction of Bcl6 expression and preferential follicular helper T (Tfh) cell fates. Varying IRF4 concentrations can affect its partitioning into distinct assemblies of DNA binding partners and DNA recognition elements that are aligned with components of distinct gene programs to effect cell fate choice, including that of Blimp-1 and Bcl6[15].

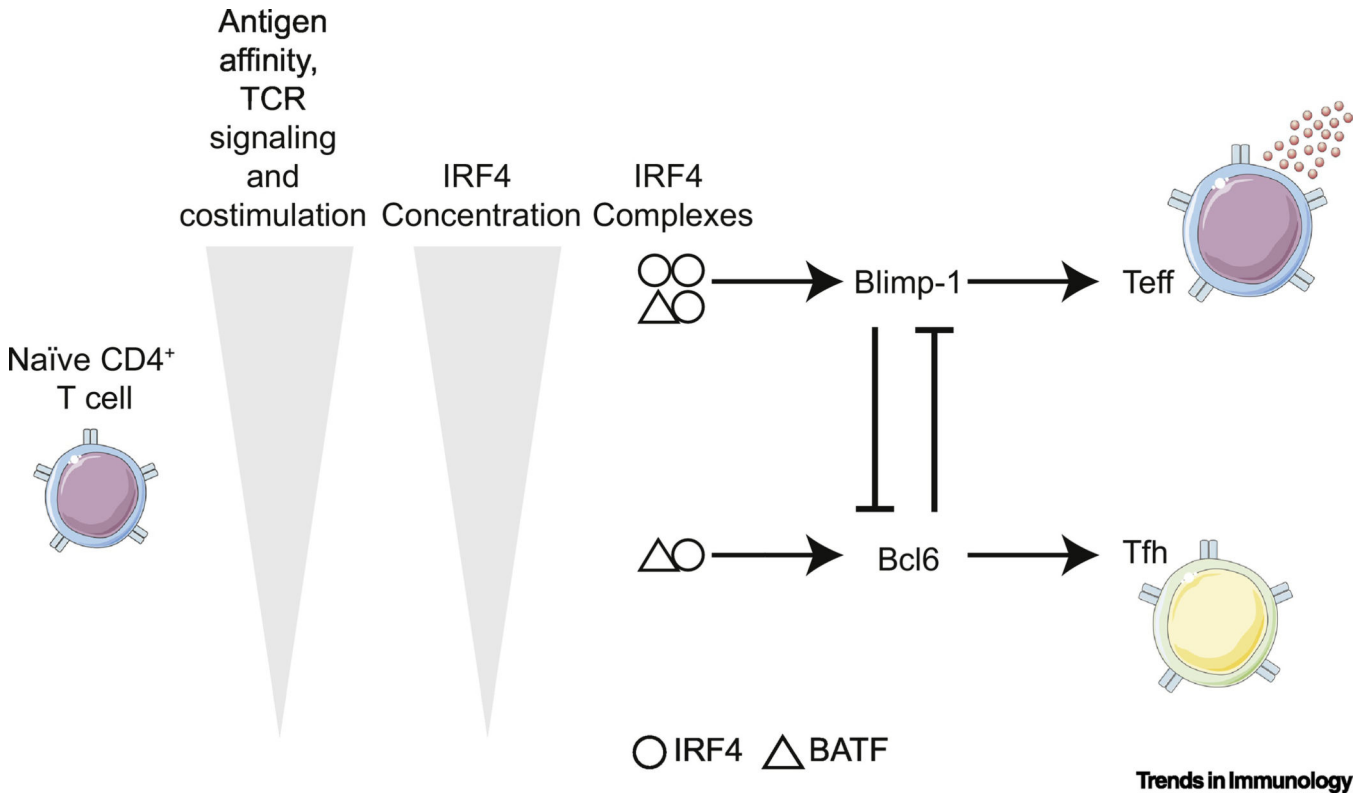
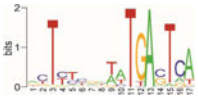
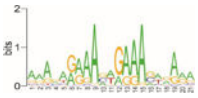


Figure 5. Antigen-Dependent Alternate CD4$^+$ T-Cell Fate Decisions.

Antigen recognition induces the expression of IRF4. Intensity of TCR signaling and costimulation scale with induced IRF4 concentrations. Higher IRF4 amounts favor the induction of Blimp-1 expression and preferential effector T-cell (Teff) fates. Alternatively, lower IRF4 amounts favor the induction of Bcl6 expression and preferential follicular helper T (Tfh) cell fates. Varying IRF4 concentrations can affect its partitioning into distinct assemblies of DNA-binding partners and DNA recognition elements that are aligned with components of distinct gene programs to effect cell fate choice, including that of Blimp-1 and Bcl6 (15]. Abbreviations: Bcl6, B-cell lymphoma 6; Blimp-1, B lymphocyte-induced maturation protein-1; IRF, interferon regulatory family.

Table 1.

IRF4 DNA binding sites and partners driving B and CD4^{+} T cell fate decisions.

DNA recognition site	Binding Partner	Relative DNA affinity	Aligned with Gene Program	Sequence Logo
EICE Ets-IRF Composite Element	PU.1 or SpiB	high	B cell GCB	
AICE AP1-IRF Composite Element	BATF/JunB or BATF/cJun	high	B cell GCB / T cell Tfh	
ZICE Ikaros-IRF Composite Element	Ikaros	high	B cell GCB	
ISRE Interferon Stimulated Response Element	IRF4 ** (homodimer)	low	B cell PC / T cell Teff	

* Currently, a comprehensive understanding of each complex's relative DNA binding efficiency is not known as only pairwise comparisons have been made. These have shown that generally, ISRE binding efficiency is substantially weaker compared to either EICE or AICE sequences[15], [16].

** The logo for AICE2 is shown. AICE1 lacks the 4 nucleotide spacer between the AP1 and IRF sites[47]. The biological basis for AICE1 and AICE2 consensus sequences has yet to be determined. Two additional variants of AICE2 have been described (not depicted). The first is composed of a partial AP-1 motif and exhibits a greater dependency on IRF4 amounts for binding [43]. The second, which is preceded by a thymidine residue 4 nucleotides upstream from the IRF site, exhibits greater IRF4 binding than a guanine variant [47]. Together, these observations raise the possibility that variations in DNA sequence binding affinities coordinated with distinct partner interactions can drive IRF4 genome localization dynamics to control cell function and alternate cell fate decisions.

*** Heterodimerization with other IRF family members is formally possible and remains to be tested. PC: plasma cells; GCB: germinal center B cells; Teff: effector T cell; Tfh: T follicular helper cell.