Published in final edited form as:

Nat Immunol. 2013 October ; 14(10): 1034–1035. doi:10.1038/ni.2709.

Fingerprinting Ikaros

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Abstract

Zinc fingers 1 and 4 of Ikaros have unique functions in the selection of Ikaros target genes, lymphocyte development and the suppression of leukemogenesis.

The Ikaros transcription factors have a broad and central role in hematopoiesis, influencing the self-renewal of hematopoietic stem cells as well as myelopoiesis and lymphopoiesis. *Ikaros* encodes multiple isoforms of Ikaros that contain as many as six C2H2-type zinc fingers; the two carboxy-terminal fingers promote protein dimerization, and the remaining four fingers contribute to DNA binding¹. Alternative splicing results in Ikaros proteins with variable numbers of zinc fingers, and while it is clear that fingers 2 and 3 are required for DNA binding, the contributions of fingers 1 and 4 to the DNA-binding specificity of Ikaros or its function *in vivo* remain poorly understood. In this issue of *Nature Immunology*, Schjerven *et al.* demonstrate that zinc fingers 1 and 4 are essential for distinct functions of Ikaros in lymphocyte development². Each finger has a substantial effect on the expression of only a small number of genes in lymphocytes that presumably underlie the critical functions of Ikaros in each of these biological processes.

Close to half of all transcription factors that have been identified in humans and about 3% of all gene products belong to the C2H2 zinc finger superfamily³. Many of those factors guide hematopoietic development in mice, including those of the GATA, Egr, Bcl-11 and Ikaros families. The number of tandemly arranged zinc fingers involved in DNA binding ranges from two to nine and, despite their structural similarities, each zinc finger domain can recognize a unique three-nucleotide sequence. Therefore, the modular zinc finger design offers many possibilities for specific DNA recognition. Ikaros is potentially capable of recognizing a 12-nucleotide sequence, yet the core DNA motif to which Ikaros binds consists of five nucleotides, GGGAA, usually followed by a pyrimidine. Binding to that core sequence requires fingers 2 and 3, which are found in all of the Ikaros DNA-binding isoforms. While the dominant Ikaros isoform has all four zinc fingers, there are naturally occurring splice variants that lack the first and/or fourth zinc fingers, and the contribution of those fingers to the selection of genes that are targets of Ikaros *in vivo* is not understood. A

prominent hypothesis, borne out by *in vitro* studies¹, is that the two flanking zinc fingers modulate the DNA-binding specificity of Ikaros. Thus, isoforms that lack zinc finger 1 or 4 could ‘preferentially’ bind to distinct subsets of Ikaros-binding sites that differ in sequences that flank the GGGAA core¹. Such observations raise the question of whether the first and fourth zinc fingers contribute to the regulation of most Ikaros-dependent genes or only a subset of those genes. Given that Ikaros binds to thousands (>7,000) of binding sites in thymocytes⁴, altered DNA-binding specificity due to changes in the number of zinc fingers could have a substantial effect on gene expression and the process of lymphocyte development *in vivo*. It is with this expectation that Schjerven *et al.* set out to examine the phenotypes of mice lacking the exons encoding the first (*Ikzf1*^{F1/F1}) or fourth (*Ikzf1*^{F4/F4}) zinc fingers of the Ikaros protein².

The functions of Ikaros in hematopoiesis have been investigated with at least three different genetic models. *Ikzf1*^{-/-} (‘Ikaros-null’) mice, which lack all Ikaros isoforms, are devoid of most adult and all fetal lymphoid lineages, including lymphoid tissue-inducing cells. However, aberrant T cell development begins shortly after birth, and these mice develop T cell lymphoma with a mean latency of approximately 3.5 months⁵. In contrast, *Ikzf1*^{DN} mice, which lack the amino-terminal zinc fingers required for DNA binding⁶, and *Ikzf1*^{plastic} mice⁷, which have a point mutation in sequence encoding the third zinc finger, show reduced DNA binding and lack all fetal and adult lymphoid lineages. The lymphoid defects of Ikaros-mutant mice emerge in the population of lymphoid-primed multipotent progenitor cells (LMPPs). LMPPs develop in these mice but fail to appropriately prime lymphoid gene expression and lack Flt3 and IL-7R, the receptors for the cytokines Flt3L and IL-7, which support further development of B cells and T cells. Even when the LMPP blockade is bypassed by experimental manipulation, to commit to the B cell fate, pro-B cells need Ikaros⁸. However, the molecular basis for the failure of lymphocyte development in *Ikzf1*^{-/-} mice remains to be fully understood, as do the functions of Ikaros in the lymphoid lineages.

The phenotypes of the *Ikzf1*^{F1/F1} and *Ikzf1*^{F4/F4} mice can best be considered in the context of the *Ikzf1*^{DN} mice, given that *Ikzf1*^{F1/F1} and *Ikzf1*^{F4/F4} mice retain the ability to interact with proteins through the carboxy-terminal zinc fingers. Strikingly, the phenotypes of the *Ikzf1*^{F1/F1} and *Ikzf1*^{F4/F4} mice do not suggest a broad decrease in the expression of all genes targeted by Ikaros. Instead, each mutation results in a distinct phenotype that is a copy of only a subset of the requirements for Ikaros (Fig. 1). The *Ikzf1*^{F1/F1} mouse has a subtly altered phenotype with a slight decline in T cell numbers; however, except for B lymphocytes, other lymphoid populations are present at nearly the numbers in wild-type mice. Although LMPPs and common lymphoid progenitors are present and express Flt3 and IL-7R, B cell numbers decrease substantially immediately after commitment, when CD19⁺ cells are beginning to express the IL-7-dependent marker BP-1 (pro-B cells). In contrast, *Ikzf1*^{F4/F4} lymphocytes progress further into the B cell lineage and outperform their wild-type counterparts at the large pre-B cell stage at which proliferation is initially driven by signals from IL-7R and the precursor to the B cell antigen receptor (BCR) before downregulation of IL-7R⁹. However, subsequent stages of B cell development are compromised by deletion of the fourth zinc finger. Moreover, *Ikzf1*^{F4/F4} mice, like *Ikzf1*^{-/-} and *Ikzf1*^{DN} mice, lack lymphoid tissue-inducer cells, natural killer cells

and Ly6C⁺ dendritic cells, as well as Flt3⁺ LMPPs and common lymphoid progenitors. T cell development at the earliest stages is also compromised, although not to the degree observed in *Ikzf1*^{-/-} or *Ikzf1*^{DN} mice. Nonetheless, *Ikzf1*^{F4/F4} mice develop T cell lymphoma with a median latency of nearly 5 months of age. Given that deletions of Ikaros are frequently associated with B cell leukemias that have translocations of the oncogenic tyrosine kinase BCR-ABL¹⁰, Schjerven *et al.* also assess the ability of Ikaros mutants lacking zinc finger 1 or zinc finger 4 to act in synergy with BCR-ABL to generate B cell leukemia². When forced to express BCR-ABL, pro-B cells with the *Ikzf1*^{F4/F4} mutation are predisposed to transformation, but those with the *Ikzf1*^{F1/F1} are not, which indicates that Ikaros zinc finger 4 specifically contributes to the Ikaros functions that prevent leukogenesis. Together these data reveal that the two zinc fingers contribute differently to Ikaros functions at distinct stages of development and in different cellular contexts.

The phenotypes of *Ikzf1*^{F4/F4} and *Ikzf1*^{F1/F1} mice suggest that zinc fingers 1 and 4 could regulate distinct sets of Ikaros target genes that are needed at different stages of development and in distinct lineages. This is a remarkable idea, given that Ikaros binds to >7,000 sites in the genome and that gene-expression studies have revealed a large number of Ikaros-dependent genes in lymphocytes^{4,11}. The expectation, therefore, would be that a large number of essential genes are regulated by Ikaros at each stage of differentiation. How could zinc finger selectivity contribute to the regulation of such a large number of genes? Interestingly, gene-expression profiling reveals that the expression of only a small number of genes is changed by more than threefold in cells with deletion of Ikaros zinc finger 1 or 4, and only a subset of those seem to be direct Ikaros targets, as revealed by chromatin immunoprecipitation followed by deep sequencing². Interestingly, the authors find differences in the binding of Ikaros zinc finger 1 or Ikaros zinc finger 4 to DNA at only a few loci, most of which include repetitive DNA elements. However, they infer some DNA-binding ‘preference’ from the data obtained by chromatin immunoprecipitation followed by deep sequencing, which shows that the Ikaros mutants bind the core consensus motif but that a longer GGGAAAAGGGAA motif is selectively absent from chromatin immunoprecipitated from *Ikzf1*^{F4/F4} thymocytes with antibody to Ikaros. These data lead the authors to propose that although Ikaros binds to many genes, only a few genes, which are modulated differently by Ikaros zinc fingers 1 and 4, are necessary for each of its critical functions². This model suggests that deregulation of only a small number of genes that are targets of Ikaros is sufficient for T cell transformation, with derepression of the cryptic internal promoter of the gene encoding the oncogenic transcription factor Notch1 being an obvious candidate for this¹². This model raises many possibilities for future investigations that should lead to a greater understanding of the mechanisms and pathways that control the development and transformation of lymphocytes.

The present study is the first *in vivo* demonstration that individual zinc fingers of a DNA-binding protein have distinct developmental functions. The data presented here are consistent with an *in vitro* analysis of the chromatin regulator CTCF showing that binding to DNA through different subsets of zinc fingers could contribute to distinct functions¹³. That model, however, had not yet been complemented by *in vivo* studies until now. Schjerven *et al.* demonstrate that mutation of sequence encoding individual zinc fingers can be a useful

strategy with which to identify key functions and targets of a multi-zinc finger transcription factor and that this approach could be applied to other essential regulators of lymphocyte development². In this case, ‘fingerprinting’ of Ikaros reveals that evolution selected for alternative usage of an optimal number of zinc fingers to control a key set of target genes that guide lymphocyte development and prevent transformation.

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Figure 1.

Distinct functions of Ikaros zinc fingers 1 and 4 in the development and transformation of lymphocytes. Comparison of the stage of the blockade in *Ikzf1*^{DN}, *Ikzf1*^{F1/F1} and *Ikzf1*^{F4/F4} mice during B cell development in the bone marrow (purple) and T cell development in the thymus (red). Blue bars indicate the abundance of cells; thicker bars indicate the stages at which Ikaros-mutant mice are susceptible to spontaneous transformation or to BCR-ABL-induced transformation. HSC, hematopoietic stem cell; CLP, common lymphoid progenitor; Imm, immature; ETP, early thymic progenitor; DN2–DN4, CD4[−]CD8[−] double-negative stages 2–4; DP, CD4⁺CD8⁺ double-positive.