HEMATOPOIESIS & STEM CELLS

Comment on Takai et al, page 3450

A new twist to the GATA switch

Sjaak Philipsen

ERASMUS UNIVERSITY MEDICAL CENTER, ROTTERDAM

In this issue of Blood, Takai et al provide some tantalizing clues on how expression of the GATA1 transcription factor, a master regulator of erythroid/megakaryocytic differentiation, is suppressed in the hematopoietic stem/progenitor cell (HSPC) compartment.

Suppression is important because forced expression of GATA1 in the stem cell compartment leads to loss of self-renewal capacity. Members of the GATA factor family of transcription factors have distinct and essential roles in hematopoiesis. GATA2 is essential for maintenance of the HSPC compartment, but it is also involved in the initial activation of GATA1 expression at the first steps of erythroid/megakaryocytic differentiation. This is referred to as the GATA factor switch. A conceptual problem of this mechanism is that GATA1 expression is suppressed in HSPCs, which are dependent on GATA2 activity. So why is GATA2 unable to activate GATA1 expression in HSPCs?

Previous work has shown that the dynamic spatiotemporal regulation of GATA factor levels is more important than their identity. When expressed under the control of the appropriate regulatory elements of the Gata1 locus, GATA2 and GATA3 transgenes rescue the lethal erythroid defects of GATA1 null mice. Thus, the developmental control mechanism of GATA1 expression is hard-wired in cis-regulatory elements residing in the Gata1 locus.

These cis-regulatory elements have been mapped in detail through analysis of reporter transgenes and also, to a more limited extent, through introduction of mutations in the endogenous Gata1 promoter. A 3.7-kb fragment in front of the hematopoietic cell–specific Gata1 gene enhancer (G1HE) is located at the promoter-distal part of this fragment, while double GATA sites and a CACC motif are found in the promoter-proximal part. All 3 elements are indispensable for erythroid cell–specific gene expression. The intervening 3.2-kb DNA fragment is of as-yet undetermined significance. Indeed, Takai et al show that combining the 3 positive regulatory elements in a 659-bp fragment is sufficient to recapitulate hematopoietic cell–specific GATA1 expression in transgenic mice. It would therefore appear that the intervening 3.2-kb DNA fragment is dispensable for appropriate regulation of GATA1 expression. However, the authors go further and discover...
that there is an unexpected twist to this story. They make full use of the green fluorescent protein (GFP) that have been inserted into the Gata1 gene to serve as a reporter for gene activation. As expected, they observe that, in the context of a wild-type Gata1 reporter transgene, expression of GFP is suppressed in the HSPC compartment.

In contrast, Gata1 reporter transgenes lacking the intervening 3.2-kb DNA fragment but retaining the 3 positive regulatory elements express GFP abundantly in this compartment. This striking difference in GFP expression is most graphically illustrated in Figure 3, panels D and F, of their article (compare wild-type transgene, G1B-GFP, with the mutant transgene, MG-GFP). This result demonstrates that the intervening 3.2-kb DNA fragment is required for suppression of the Gata1 gene in the HSPC compartment. But how is this achieved?

A survey of epigenetic modifications has revealed that the Gata1 locus is heavily methylated in the HSPC compartment. DNA methylation, occurring at cytosine residues in the dinucleotide sequence 5’-CG-3’, is a very stable modification which is strongly associated with repression of gene activity. Takai et al analyze DNA methylation of the transgenes in HSPCs. In the case of the wild-type transgene, they find high methylation levels at the Gata1 promoter and regulatory elements. In contrast, DNA methylation was much reduced in the mutant transgene lacking the intervening 3.2-kb DNA fragment. This fragment is therefore required for high DNA methylation levels of critical Gata1 regulatory elements in HSPCs. Hypermethylated DNA attracts repressor proteins including DNA methyltransferase 1 (DNMT1). DNMT1 ensures that DNA methylation patterns are faithfully passed on to the daughter cells after cell division, thus locking the Gata1 gene stably in an inactive configuration. The authors provide evidence that under these conditions GATA2 is unable to bind to the Gata1 regulatory elements, providing a mechanistic explanation for the inability of GATA2 to activate the Gata1 gene in HSPCs (see figure). To proceed to lineage commitment and differentiation would require demethylation of the Gata1 locus, and it remains to be investigated how this is achieved. This could be through a passive process, involving failure to maintain methylation after DNA replication.

Interestingly, Takai et al characterize a binding site for E2F transcription factors which provides a potential lead. In quiescent cells, E2F factors form a repressive complex including the Retinoblastoma protein and DNMT1. When the cells enter the cell cycle, the Retinoblastoma protein is phosphorylated and the repressive complex is released, allowing E2F to become active. If the repressive complex is not reformed after cell division, DNMT1 would fail to maintain DNA methylation and the methylation marks would be lost. Alternatively, demethylation might be an active process, for which a number of different mechanisms have been proposed including oxidation of the methyl groups by TET proteins, modification by AID/APOBEC enzymes, and base excision DNA repair. Aberrant DNA methylation is a hallmark of many cancers including hematologic malignancies, and DNA methylation inhibitors are used to treat patients with myelodysplastic syndromes.

Finally, the current study relies on the use of transgenes, which are analyzed in the presence of the endogenous Gata1 locus. An important next step would be to assess the role of the elements identified in the context of the endogenous locus. Although this used to be a mammoth task, the flurry of recent articles describing CRISPR/CAS9 as a very efficient tool for mammalian genome engineering makes this a realistic proposition. Such experiments will provide an increasingly detailed picture of the dynamic regulation of endogenous GATA factor expression during hematopoiesis.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

A new twist to the GATA switch

Sjaak Philipsen