MYELOID NEOPLASIA

Comment on Trinh et al, page 1331, and van der Kouwe et al, page 1345

To switch or not to switch: PU.1 expression is the question

Julie A. I. Thoms,[1] John E. Pimanda,[1,2] and Olaf Heidenreich[3] | [1]University of New South Wales Sydney; [2]Prince of Wales Hospital; [3]Princess Maxima Center for Pediatric Oncology

In this issue of *Blood*, articles by van der Kouwe et al[1] and Trinh et al[2] characterize the complex regulation of PU.1 (*SPI1*) expression involving the interplay of a single enhancer with the canonical sense and the alternate antisense promoter.

The transcription factor PU.1 is a master hematopoietic regulator involved in hematopoietic stem cell maintenance and myeloid and B lymphoid lineages.[3,4] PU.1 does not function as an on/off switch; instead, graded expression levels determine its precise role in specific cell types,[5] and expression levels must be tightly controlled throughout development. Levels of PU.1 increase during myelopoiesis,[6] but expression is reduced in a range of acute myeloid leukemia (AML) cells, particularly those with core binding factor (CBF) fusions [RUNX1-ETO caused by t(8;21) or CBFβ-MYH11 caused by inv(16)].[4,7] Reduced expression, but not complete ablation, in mice leads to AML.[6] Conversely, reexpression of PU.1 can cause AML cells to differentiate.[7] A key PU.1 upstream regulatory element (URE) is located upstream of the sense promoter (proximal promoter [PrPr]), and this can be positively regulated by RUNX1, which binds directly to the URE[4,8-10] (see figure, panel A). PU.1 expression can be negatively regulated by a long noncoding RNA (lncRNA) (PU.1 antisense RNA [asRNA]), which reduces both PU.1 messenger RNA (mRNA) and the protein and is initiated by an antisense promoter (AsPr) located in exon 3[11] (see figure, panel A). As with the PrPr, AsPr interacts with and is regulated by the URE. However, the mechanisms of spatiotemporal control of *PU.1* expression are not completely resolved.

In their article, van der Kouwe et al find that the ratio of PrPr to AsPr chromatin accessibility and transcriptional activity changes during development, with increased PrPr accessibility and PU.1 mRNA expression in the myeloid and B lymphoid lineages. In contrast, high AsPr accessibility precedes complete shutdown of the *PU.1* locus during T-cell maturation. Because AsPr and PrPr both loop with and are regulated by the URE, the authors then investigated chromatin interactions at the *PU.1* locus. In the T-lymphoid context, the URE is predominantly looped with AsPr with a concomitant high asRNA:mRNA ratio (see figure, panel B), whereas in myeloid cells, the URE interacts predominantly with PrPr, which favors sense transcript expression (see figure panel C). Interestingly, RUNX1 binds to the URE and both PrPr and AsPr, which suggests that it controls *PU.1* expression during hematopoiesis by modulating the ratio of sense to antisense transcription.

Precisely how does RUNX1 occupation affect looping between URE and the promoters? The study by Trinh et al provides complementary new insights. They screened for RUNX1-interacting RNAs and identified a polyadenylated lnc-RNA termed *LOUP* that is myeloid specific and originates from the URE. *LOUP* facilitates chromatin loop formation between the URE and PrPr by recruiting RUNX1 to both elements thereby promoting *PU.1* expression and, ultimately, myeloid differentiation with concomitant inhibition of proliferation (see figure, panel C).

Given the known roles of PU.1 and RUNX1 in AML, both studies focused on PU.1 regulation in leukemia. Similar to wild-type RUNX1, the CBF fusion proteins RUNX1-ETO and CBFβ-MYH11 are able to transactivate AsPr, which suggests the intriguing possibility that PU.1 perturbation in CBF leukemias might be mediated by AsPr. In line with this finding, CBF AMLs have increased AsPr accessibility and asR-NA expression compared with AMLs with a normal karyotype. Furthermore, RUNX1-ETO also binds to the URE and diminishes *LOUP* expression. Knockdown of RUNX1-ETO, which also induces a more mature myeloid phenotype, increases *LOUP* levels, reduces the *PU.1* asRNA:mRNA ratio, and promotes interaction between URE and PrPr (see figure, panel D). Thus, in RUNX1-ETO AML, *PU.1* expression is actively repressed by a chromosomal structure that is unfavorable to interaction of the URE with the sense promoter and enhances expression of an antisense transcript that is known to repress *PU.1*. Furthermore, RUNX1-ETO–mediated repression of the *PU.1* locus seems to require active maintenance, similar to other reports of RUNX1-ETO–induced chromatin remodeling.[12] The fact that *PU.1* repression by this mechanism is reversible may be an opportunity for therapeutic targeting.

Key findings of these studies are: (1) that PU.1 is regulated by competition between a coding and a noncoding promoter for interaction with the enhancer, (2) that an enhancer-driven lncRNA promotes looping with the sense promoter, and (3) that CBF AMLs can co-opt these 2 mechanisms to reduce *PU.1* expression. Questions remain regarding precisely how the RUNX1-ETO fusion protein mediates altered chromatin looping and whether this is an immediate consequence of fusion gene expression or requires previous chromatin remodeling or destabilization. RUNX1-ETO alters global chromatin conformation and promoter-enhancer interactions.[12] One possibility is that the global effects of fusion gene expression favor a chromatin conformation in which RUNX1-ETO can bind AsPr and modify its chromatin interactions. Alternatively, RUNX1-ETO might directly promote URE-AsPr looping by homodimerization or interaction with other factors bound to the URE or AsPr. It also remains unclear whether PU.1 promoter competition is a mechanism for PU.1 downregulation in other AML subtypes, or indeed whether similar regulatory modules are used by other genes. In this context, it is interesting to note that multiple established and potential therapeutic targets such as *CCND2*, *CDK6*, and *WT1* show arrangements of sense and antisense promoters that are controlled by leukemic fusion proteins.[13]
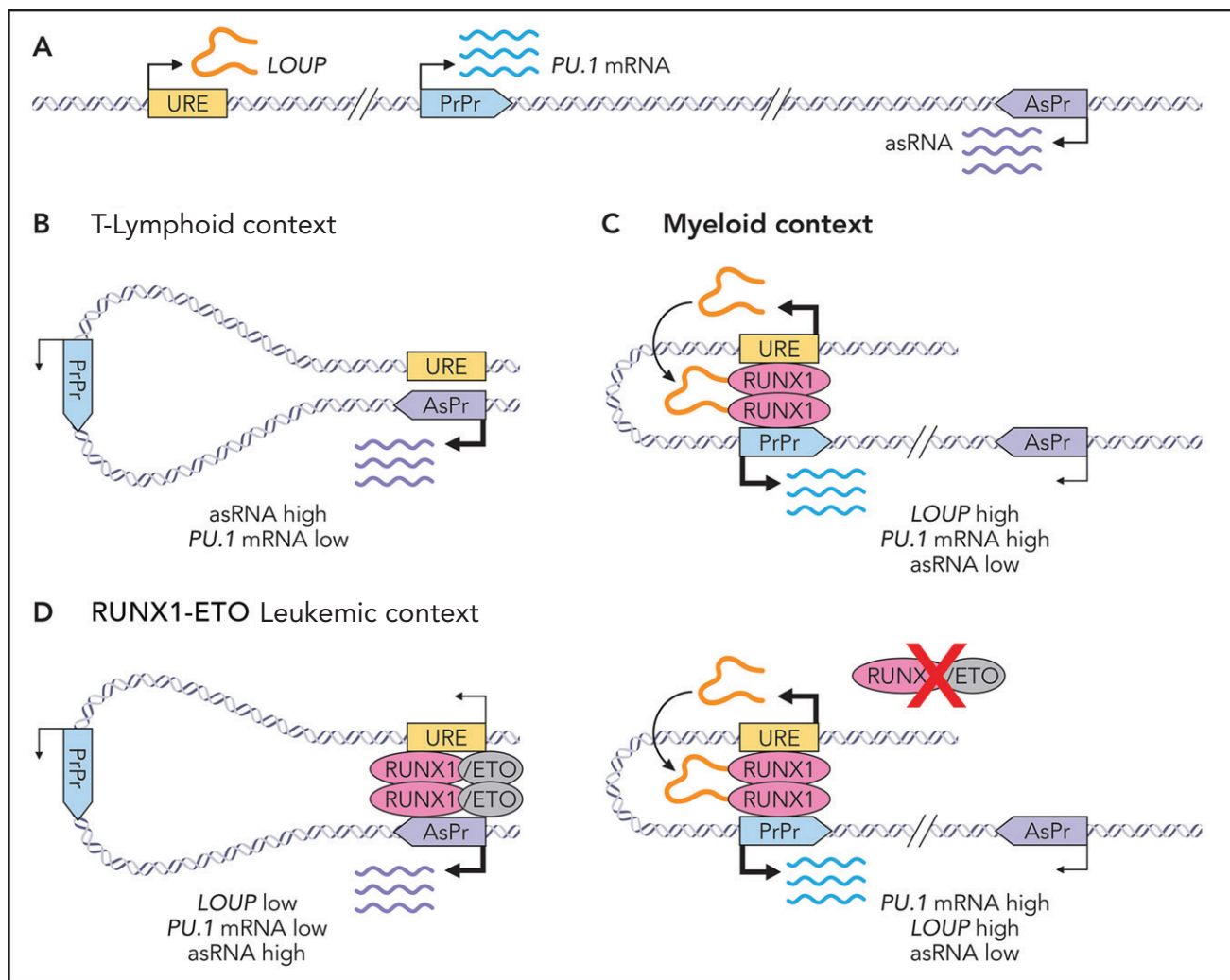
The *PU.1* (*SPI1*) locus in healthy hematopoiesis and the RUNX1-ETO leukemic context. (A) Representation of the *PU.1* (*SPI1*) locus showing the URE regulatory element, the coding promoter (PrPr), the antisense promoter (AsPr), and RNAs originating from each (*LOUP* [orange] from the URE, *PU.1* coding mRNA from PrPr [blue], and asRNA from AsPr [purple]; arrows indicate transcription initiation sites). Both PrPr and AsPr can be regulated by URE. (B-C) *PU.1* locus conformation in healthy cells. T-lymphoid context: URE interacts with AsPr (B), and myeloid context: URE interacts with PrPr (C). Loop formation is mediated by RUNX1 (pink) and *LOUP*. (D) *PU.1* locus conformation in RUNX1-ETO AML. RUNX1-ETO (pink/gray) mediates URE interaction with AsPr, leading to reduced *PU.1* mRNA expression. Depletion of RUNX1-ETO leads to restoration of *LOUP* expression, interaction of the URE with PrPr, and increased *PU.1* mRNA expression. Arrow thickness in panels B-D indicates relative transcriptional output from URE, PrPr, and AsPr. Professional illustration by Patrick Lane, ScEYEnce Studios.

These studies extend our understanding of how *PU.1* expression is tightly controlled during normal hematopoiesis and add to the increasing catalog of epigenetic mechanisms by which oncogenes perturb normal gene regulation. The hope is that these collective insights will reveal leukemia vulnerabilities and point to new therapeutic possibilities for AML.

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REFERENCES

1. van der Kouwe E, Heller G, Czibere A, et al. Core-binding factor leukemia hijacks T-cell prone PU.1 antisense promoter. *Blood.* 2021;138(16):1345-1358.

2. Trinh BQ, Ummarino S, Zhang Y, et al. Myeloid lncRNA *LOUP* mediates opposing regulatory effects of RUNX1 and RUNX1-ETO in t(8;21) AML. *Blood.* 2021;138(16):1331-1344.

3. Iwasaki H, Somoza C, Shigematsu H, et al. Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation. *Blood.* 2005;106(5):1590-1600.

4. Staber PB, Zhang P, Ye M, et al. The Runx-PU.1 pathway preserves normal and AML/ETO9a leukemic stem cells. *Blood.* 2014;124(15):2391-2399.

5. DeKoter RP, Singh H. Regulation of B lymphocyte and macrophage development by graded expression of PU.1. *Science.* 2000;288(5470):1439-1441.

6. Rosenbauer F, Wagner K, Kutok JL, et al. Acute myeloid leukemia induced by graded reduction of a lineage-specific transcription factor, PU.1. *Nat Genet.* 2004;36(6):624-630.

7. Vangala RK, Heiss-Neumann MS, Rangatia JS, et al. The myeloid master regulator transcription factor PU.1 is inactivated by AML1-ETO in t(8;21) myeloid leukemia. *Blood.* 2003;101(1):270-277.

8. Huang G, Zhang P, Hirai H, et al. PU.1 is a major downstream target of AML1 (RUNX1) in adult mouse hematopoiesis. *Nat Genet.* 2008;40(1):51-60.

9. Ptasinska A, Assi SA, Mannari D, et al. Depletion of RUNX1/ETO in t(8;21) AML cells leads to genome-wide changes in chromatin structure and transcription factor binding. *Leukemia.* 2012;26(8):1829-1841.

10. Beck D, Thoms JA, Perera D, et al. Genome-wide analysis of transcriptional regulators in human HSPCs reveals a densely interconnected network of coding and noncoding genes. *Blood.* 2013;122(14):e12-e22.

11. Ebralidze AK, Guibal FC, Steidl U, et al. PU.1 expression is modulated by the balance of functional sense and antisense RNAs regulated by a shared cis-regulatory element. *Genes Dev.* 2008;22(15):2085-2092.

12. Ptasinska A, Pickin A, Assi SA, et al. RUNX1-ETO depletion in t(8;21) AML leads to C/EBPα- and AP-1-mediated alterations in enhancer-promoter interaction. *Cell Rep.* 2019;28(12):3022-3031.e7.

13. Martinez-Soria N, McKenzie L, Draper J, et al. The oncogenic transcription factor RUNX1/ETO corrupts cell cycle regulation to drive leukemic transformation. *Cancer Cell.* 2018;34(4):626-642.e8.