Linking histone methylation, transcription rates, and stem cell robustness

Justin C. Wheat[1] and Ulrich Steidl[1,2,3,4]

[1]Department of Cell Biology; [2]Department of Medicine; [3]Albert Einstein Cancer Center and [4]Ruth L. and David S. Gottesman Institute for Stem Cell Research and Regenerative Medicine, Albert Einstein College of Medicine - Montefiore Medical Center, Bronx, NY, USA

E-mail: ulrich.steidl@einstein.yu.edu

doi:10.3324/haematol.2018.196089

As blocked differentiation is a hallmark of most tumors, significant efforts have been made to understand the regulation of gene networks during normal differentiation and how perturbation of these regulatory processes contribute to tumor initiation. An emerging question of interest within this arena is how the core transcriptional machinery and the epigenome interact during transcription. While strong correlations between certain histone marks and transcriptional activity have been found, critical questions remain, including the information encoded in these marks, whether these marks are cause or consequence of polymerase activity, and what functional cost is incurred by stem cells when these marks are perturbed.

In this issue of the Journal, Zhou *et al.*[1] study the hematopoietic stem cell defects associated with genetic deletion of the mammalian H3K36 tri-methyltransferase, *Setd2*. While embryonic deletion of *Setd2* within the endothelial/hematopoietic lineages by a *Tie2-Cre* is lethal, deletion with *Vav* and the inducible *Mx1-Cre* systems permitted the study of this critical enzyme during hematopoiesis. The Authors found significant multilineage leukopenia, multiple erythroid dysplasias, and ultimately bone marrow failure in knockout mice. Interestingly, this study found an expansion of early erythroblasts and mature megakaryocytes in the bone marrow, and a macrocytic anemia with thrombocytosis in the peripheral blood, indicating that the erythroid/megakaryocytic lineage specification is at least partially maintained despite *Setd2* loss. Indeed, the putative number of preCFU-E (as defined by FACS) and BFU-E (defined by colony assay) were increased in *Setd2* knockout mice, indicating that the peripheral anemias do not arise from a reduction in progenitor cells committing to the erythroid lineage. Characterization of the HSC compartment in these mice also demonstrated substantial defects in repopulating capacity in transplantation experiments and suggest that the erythroid-megakaryocytic bias of *Setd2* KO cells is intrinsic to HSC. Finally, *Setd2* HSC were found to cycle more than wild-type HSC and were more sensitive to challenge with 5-FU. In total, *Setd2* appears to play an essential role in normal HSC biology.

Next, Zhou *et al.*[1] established the molecular mechanism underlying these dramatic phenotypic findings. First, they found significant changes in both the mRNA and protein levels of other H3K36 methyltransferases, with *Ash1l* decreasing and *Nsd1/2/3* increasing. As these enzymes catalyze the mono- and di-methylation reactions on H3K36, these authors speculated that *Nsd* enzymes may compete with *Setd2* at H3K36. In cell lines, they found that overexpression of *Nsd* proteins phenocopied the molecular aberrations seen with *Setd2* loss of function, consistent with a mutual antagonism between these methyltransferases. Moreover, perturbation of the mono/di- to tri-methylation status of H3K36 also lead to changes in other histone marks correlated with active transcription, such as increased H3K79me2 and H3K4me3, and decreased H3K27me3. As both H3K36 and H3K79 have been strongly linked to polymerase processivity, Zhou *et al.*[1] looked at the distribution and abundance of initiated and actively elongating polymerase, indicated by the presence of phosphorylation marks on the CTD at serine 5 and serine 2, respectively. Intriguingly, these Authors show robust increases in the Ser2P after deletion of *Setd2*, as well as increased ChIP signal within the exonic region of elongation complex sensitive genes such as *Mycc*. These changes in polymerase processivity led to specific changes in gene expression, with increased expression of terminal erythroid genes such as *Gata1*. Finally, pharmacological inhibition of other super elongation complex enzymes, including DOT1L, BRD4, and CDK9, fully reversed the transcriptional and phenotypic changes observed in *Setd2* knockout HSC.

Prior work integrating Global Run-On sequencing (Gro-Seq) and ChIP-seq of H3K36me3 has demonstrated substantial anti-correlation between this histone mark and polymerase elongation rates.[2] The study by Zhou *et al.*[1] is, to our knowledge, the first to demonstrate *in vivo* evidence for a directional and putatively causal link of this mark with polymerase kinetics. Moreover, this study provides significant evidence for the importance of *Setd2*, and consequently H3K36me3, to hematopoietic stem cell function. A growing body of literature has demonstrated that the core transcriptional machinery is not a simple digital switch responding to upstream interactions of transcription factors, but rather a highly regulated, multi-step process that behaves differently within different cells and in different contexts (reviewed by Coulon *et al.*,[3] Goodrich and Tjian,[4] and Jonkers and Lis[5]). The study by Zhou *et al.*[1] confirms those results in the context of hematopoiesis and stem cell biology, as well as prompts a number of important questions that must now be resolved. Why are erythroid cells more robust to changes in *Setd2* activity? What facilitates crosstalk between the CTD and H3K36 methylation? What biophysical parameters change to increase transcription elongation, and how does polymerase "read" differences in K36 methylation status? And how do different genes differentially respond to perturbations in this single mark? While many questions remain, this study by Zhou *et al.*[1] provides substantial impetus for the continued exploration of the fundamental questions surrounding the core transcriptional machinery, its tissue-specific regulation, and its role in modulating stem cell function.

References

1. Zhou Y, Yan X, Feng X et al. Setd2 regulates quiescence and differentiation of adult hematopoietic stem cells by restricting RNA polymerase II elongation. Haematologica. 2018;103(7):1110-1123.
2. Jonkers I, Kwak H, Lis JT. Genome-wide dynamics of Pol II elongation and its interplay with promoter proximal pausing, chromatin, and exons. Elife. 2014;3:e02407.
3. Coulon A, Chow CC, Singer RH, Larson DR. Eukaryotic transcriptional dynamics: from single molecules to cell populations. Nat Rev Genet. 2013;14(8):572-584.
4. Goodrich JA, Tjian R. Unexpected roles for core promoter recognition factors in cell-type-specific transcription and gene regulation. Nat Rev Genet. 2010;11(8):549-558.
5. Jonkers I, Lis JT. Getting up to speed with transcription elongation by RNA polymerase II. Nat Rev Mol Cell Biol. 2015;16(3):167-177.