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Checks and Balances: Rpd3 Issues Executive Orders in Developmental Enhancer Regulation

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Stem cells use poised enhancers of developmental regulators to maintain pluripotency and for subsequent activation in differentiating progeny. In this issue of *Developmental Cell*, Janssens et al. (2017) demonstrate that the *erm* enhancer is maintained in a poised state in neural stem cells by the histone deacetylase Hdac1/Rpd3.

Biological differences between cell types arise in large part due to differential patterns of gene expression. Studies in recent years have demonstrated that a large amount of the genome is dedicated to making sure that specific genes are expressed in the right place at the right time. These gene-distal sequence elements, termed enhancers, are key contributors to gene regulation, allowing for both spatial and temporal control of gene expression during organism development. Genome-wide studies suggest that active enhancers exhibit distinctive chromatin "signatures," including mono- and dimethylation of histone 3 on lysine 4 (H3K4me1/2) and acetylation of a number of histone lysine residues, including H3K27 (H3K27ac) (reviewed in Calo and Wysocka, 2013). Surprisingly, these studies demonstrated that developmentally regulated enhancers in pluripotent cells can exist in a "poised" state in which the enhancer is lacking H3K27ac and is instead enriched with H3K27me3 (Rada-Iglesias et al., 2011; Zentner et al., 2011). These enhancers are able to drive gene expression during differentiation coincident with loss of H3K27me3 and gain of H3K27ac. Intriguingly, poised regions remain bound by the histone acetyltransferase p300 in the pluripotent state, suggesting the existence of a mechanism to prevent premature enhancer acetylation and activation. In this issue of *Developmental Cell*, Janssens et al. suggest a novel mechanism by which the histone deacetylase Hdac1/Rpd3 maintains the poised chromatin state of a developmentally regulated enhancer in neuronal stem cell development in the fly larval brain. Loss of additional transcriptional repressors relieves Rpd3-mediated enhancer repression, allowing cells to rapidly transition from a stem cell to progenitor state.

Much of what we know regarding enhancer function has been learned using *Drosophila* genetics. Janssens et al. (2017) take advantage of both the genetic and developmental potential of this system to study the activation of a single enhancer during the differentiation of neural stem cells (type II neuroblasts) to immature intermediate neural progenitors (INPs) in *Drosophila* larvae. This well-characterized and rapid transition is marked by downregulation of self-renewal-promoting transcriptional repressors and upregulation of the transcription factor *earmuff* (*erm*) to restrict the developmental potential of INPs (Homem et al., 2015). To determine how *erm* transcription is regulated during this process, the authors identified a minimal *erm* enhancer fragment (250 bp) that was silent in type II neuroblasts but able to drive expression of a reporter transgene in immature INPs. The rapid appearance of *erm*-driven expression within 2 hr of immature INP birth led the authors to hypothesize that the *erm* enhancer may be poised for activation in type II neuroblasts. Chromatin immunoprecipitation experiments demonstrated that the endogenous *erm* enhancer is indeed enriched with both H3K4me2 and H3K27me3, indicative of the poised state, in type II neuroblasts. Surprisingly, the minimal *erm* enhancer in type II neuroblasts was enriched with H3K4me2, but not H3K27me3.

There are several possible mechanisms that might support the chromatin signature observed at poised enhancers (Figure 1). For example, the complex recruited to these regions of chromatin may lack a co-activator required for p300 enzymatic activity or, alternatively, may contain a direct inhibitor of p300 activity. Additionally, the presence of H3K27me3, installed by the PRC2 complex (Blackledge et al., 2015), might prevent enhancer activation, either through its mutually exclusive relationship with H3K27ac or through H3K27me3-mediated recruitment of the PRC1 complex that might alter local chromatin structure and accessibility. Alternatively, active turnover by deacetylases may keep poised enhancers from premature activation by keeping acetylation levels in check.

Based on their observation that the minimal *erm* enhancer remained inactive in type II neuroblasts while lacking H3K27me3, Janssens et al. (2017) tested whether the PRC2 complex was required to prevent premature *erm* expression. In line with their observation of chromatin state, neither loss of Suz12 nor loss of Ezh2, two critical components of the PRC2 complex, resulted in *erm* enhancer activation in type II neuroblasts. Instead, loss of the histone deacetylase Rpd3 led to premature activation of the *erm* enhancer, as evidenced by Erm expression in type II neuroblasts and their premature differentiation. These data support a model whereby the poised *erm* enhancer is kept in this state through constant deacetylation of the regulatory element. The authors went

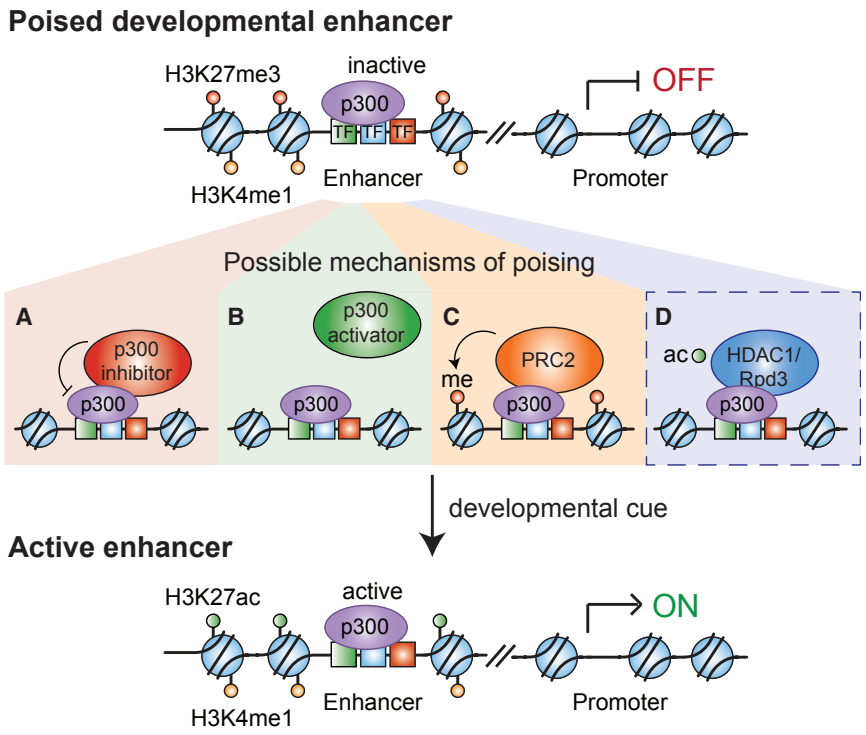

Figure 1. Possible Mechanisms of Maintaining Poised Enhancers
Poised enhancers, marked by H3K4me1, H3K27me3, and the presence of p300, might be maintained in an inactive state by (A) a direct p300 inhibitor, (B) the absence of a p300 co-activator, (C) antagonistic PRC2 activity, or (D) continual "decommissioning" by a histone deacetylase, the model supported herein.

on to identify Tip60 and HBO1 as histone acetyltransferases required for the differentiation to immature INPs. As these acetyltransferases' main histone substrates are H2A and H4 (Lee and Workman, 2007), respectively, these observations would invoke a mechanism by which these acetylation events, or perhaps even non-histone acetylation, may play a more prominent role in enhancer activation than H3K27ac.

While the proposed model of deacetylase-mediated regulation is compelling, it is important to note that the data presented here do not rule out other mechanisms that might regulate enhancer activation. Critically, the minimal *erm* enhancer lacks genomic context that might provide additional means of regulation. Additionally, a direct link between loss of Rpd3 activity, activation of Tip60 and HBO1, and acetylation of the enhancer has not been established. This work would be strengthened by additional mechanistic analysis of the endogenous enhancer at different developmental stages and by extending this analysis to other developmentally regulated enhancers.

In spite of recent advances in our ability to discover genome-wide enhancers based on chromatin signatures and other distinctive features, it has been difficult to directly test the functional impact of the chromatin modification state of enhancers in a developmental context. This study from Janssens et al. (2017) adds significantly to our understanding of the mechanism by which a poised enhancer can be developmentally licensed to activate gene expression and cell fate commitment. Once activated in INPs, Erm restricts developmental potential through a negative feedback loop by repressing the expression of genes that promote type II neuroblast self-renewal. This work paves the way for a host of exciting questions. Specifically, is Rpd3 recruited to the *erm* enhancer? And if so, by what mechanism? How is Rpd3 function at enhancers delineated from its function at genes? What is the acetylation landscape of this region upon differentiation from type II neuroblasts to INPs? What is the mechanism that prevents Rpd3 activity at the *erm* enhancer in INPs? More globally, is this mechanism of deacetylase decommissioning specific to this enhancer during this developmental transition, or is it a more general mechanism of regulating poised enhancers genome-wide and under many developmental or biological contexts? What is the role of acetylation in enhancer activation, and is it specific to particular histone lysine residues? Further, how are all the regulatory inputs required for developmental cell fate specification integrated through enhancer function and subsequent gene regulation? With the advent of new tools to manipulate genomes, including enhancer elements (Fulco et al., 2016; Sanjana et al., 2016), we look forward to discovering the answers to these questions and more.

In sum, this new study sheds light on a mechanism regulating the "dark matter" of our DNA, and it will certainly inform future investigation of enhancer function.

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