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Keep them close: PRC2 poises enhancer-promoter interactions at anterior neuronal genes

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Abstract

Enhancers exist in different epigenetic states: active, primed, or poised. However, it is not yet understood how the different enhancer states influence gene activation. In this issue of Cell Stem Cell, Cruz-Molina et al. (2017) unravel how poised enhancers activate anterior neural genes and the role of Polycomb proteins in enhancer-promoter contacts.

Main Text

Enhancers are regulatory regions important for gene expression: when active, they promote the transcription of their target genes, in a mechanism that involves the binding of transcription factors and recruitment of the transcription machinery. Enhancers can be located very far from their target genes and contact them through chromatin looping (reviewed in (Beagrie and Pombo, 2016). Looping involves the action of structural protein complexes such as cohesin, CTCF, and Mediator, which bring together genes and enhancers in 3D space, despite their linear distance along the genome.

Enhancers are cell-, time- and stimulus-specific and can be found in different epigenetic states: active, primed, or poised (Calo and Wysocka, 2013; Creyghton et al., 2010; Rada-Iglesias et al., 2011; Zentner et al., 2011). Active enhancers induce strong expression of target genes and are enriched in histone modifications such as H3K27ac and H3K4me1, and they are bound by the histone acetyltransferase P300. In contrast, primed enhancers are not enriched for H3K27ac, but retain H3K4me1 and P300 and drive basal levels of gene activation. Lastly, poised enhancers are marked by the canonical markers of active enhancers, H3K4me1 and P300, but also by the repressive histone mark H3K27me3, associated with Polycomb Repressive Complex 2 (PRC2) silencing. Poised enhancers were first described in human and mouse embryonic stem cells (ESCs), were found to target important genes (which are inactive in ESCs), and were activated upon differentiation. During activation, poised enhancers lose H3K27me3 and acquire acetylation at the same residue, changing from poised to active states. Early studies from Rada-Iglesias and colleagues showed that poised enhancers can acquire H3K27ac during

differentiation to become active (Rada-Iglesias et al., 2011). While the activity and regulation of active enhancers has been the target of intense study, how poised enhancers act to regulate gene expression remains unclear.

Polycomb repressive complexes (PRCs) are epigenetic regulators important for gene repression. PRC1 monoubiquitylates histone H2A tail on residue K119, and PRC2 methylates histone H3 tail on residue K27, producing H2AK119ub1 and H3K27me3, respectively. Both histone marks have roles linked to chromatin compaction and gene silencing. PRCs are increasingly thought to have roles as organizers of chromatin architecture, revealing a new layer of Polycomb-mediated regulatory mechanisms. For example, they have been shown to drive interactions between regulatory elements, such as enhancer and promoters, and to create larger-scale Polycomb domains (Entrevan et al., 2016).

In this issue of Cell Stem Cell, Cruz-Molina and colleagues (2017) show how PRC2 mediates contacts between poised enhancers and genes which are still silenced in ESCs. These contacts create a permissive environment that becomes important for gene activation during anterior neuronal differentiation (Figure 1). To identify poised enhancers, the authors mapped genome-wide P300 and H3K27me3 in mouse ESCs. They identified around 1,000 poised enhancers, the vast majority of which overlap with active enhancer regions in brain tissues. Through the combination of *in vitro* differentiation, 3D chromatin contact mapping, CRISPR-Cas9 and PRC2 knockouts, Cruz-Molina and coauthors investigated how poised enhancers influence gene expression during cell differentiation. Intriguingly, they observed that poised enhancers are in physical contact with their target promoters already in ESCs, before genes and enhancers become active in anterior neuronal progenitors (aNPCs). When poised enhancers are deleted, the activation of their target genes in aNPCs is disrupted. A detailed investigation of a specific poised enhancer deletion showed decreased recruitment of RNA polymerase II (RNAPII) at a target gene promoter in aNPCs.

To investigate the role of Polycomb in the enhancer-promoter interaction, Cruz-Molina and colleagues also took advantage of knockouts of two components of PRC2 complex: EED and SUZ12. Noticeably, in the absence of PRC2, the contacts between genes and poised enhancers are disrupted; PRC2 itself or its associated mark, H3K27me3, seem to be important for these interactions. Known structural proteins involved in looping, such as CTCF, Mediator, and cohesin, were not found enriched at the base of contacting loops, suggesting that the poised enhancer-promoter interactions are dependent on PRC2.

Poised enhancers reside in regions rich in CpG islands, which are genomic elements that have been previously associated with preferred Polycomb activity. The authors speculate that CpG islands might facilitate H3K27me3 deposition and enhancer regulation. Interestingly, after PRC2 knockout, cells were differentiated to aNPCs

poised enhancers no longer acquired H3K27ac enrichment, despite lacking H3K27me3. PRC2 modification or associated mechanisms appear therefore to work in ESCs not only by inhibition of premature gene activation, but also as part of the mechanisms that lead to future activation of enhancers.

The work of Cruz-Molina et al. (2017) sheds a new light on the role of Polycomb in gene and enhancer regulation that expands beyond gene silencing, and it opens new questions on the mechanisms of Polycomb-mediated looping and transcriptional tuning. While this study focuses on poised enhancers almost exclusively related to anterior neuronal differentiation, other poised enhancers might be activated in different cell lineages or at later time points, in a more widespread mechanism of enhancer poising. A better understanding of the dynamics and nature of Polycomb-mediated contacts will help dissect the mechanism behind this mode of gene regulation. Genome-wide analyses focused on Polycomb-mediated contacts will also help clarify how contacts spread across the genome, when more than one poised enhancer regulates the same gene, and whether they act in concert with enhancers in other states of activation (active, poised, or primed). It will also be interesting to explore whether poised enhancers regulate only genes, or also other enhancers (for example in PRC1-mediated hubs, as suggested in Schoenfelder et al., 2015). Cruz-Molina and colleagues also show that poised enhancers can reside in enhancer-dense regions, which have been called super-enhancers. Polycomb may play important roles within super-enhancer hubs, not only by regulating the on/off switching of specific genes, but also by fine-tuning other active or poised enhancers. Follow-up studies will be needed to identify the specific players involved in the recognition and contacts between poised enhancers and their target genes in ESCs, and how this binding evolves when cells differentiate into other lineages and genes become silenced or activated. Changes in transcription factor binding may play a role in the switch from methylation to acetylation of H3K27 in neuronal and potentially other cell lineages. Lastly, as RNAPII occupies Polycomb-repressed developmental genes in ESCs (Brookes et al., 2012), it will be important to understand whether poised RNAPII also occupies poised enhancers and its role in the mechanisms that lead to the induction of neuronal genes.

Rada-Iglesias and colleagues show how epigenetic regulators and chromatin architecture work together to fine-tune gene activation mechanisms during differentiation. Their work describes a new layer of regulation in which Polycomb plays a central and active role in the mechanisms of cell-type-specific gene activation through sustaining enhancer-promoter interactions in a poised environment.

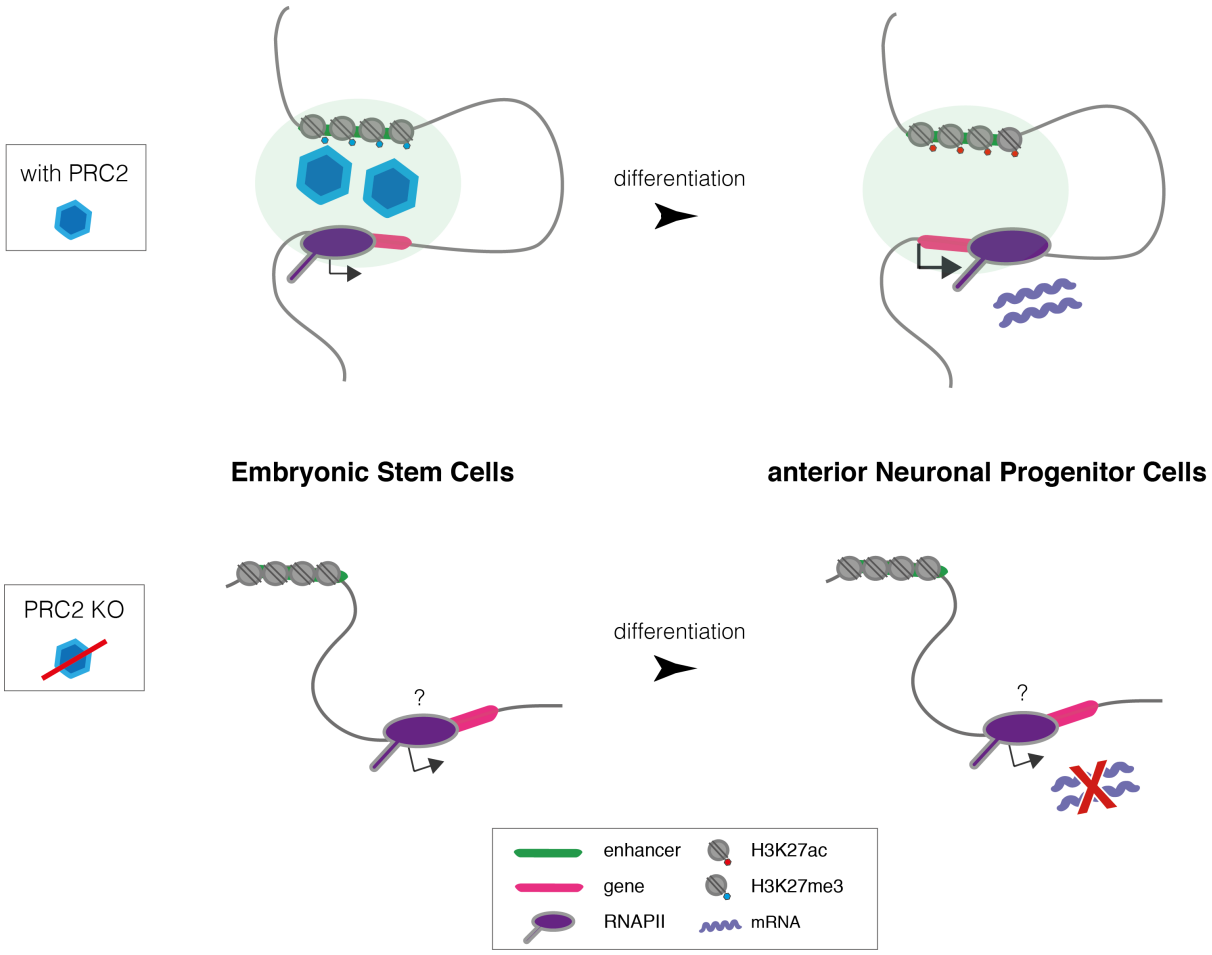

Figure 1
Schematic of regulation of gene activation through poised enhancers during anterior neuronal differentiation

Above: Poised enhancers in mouse ESCs are already in contact with their target genes. Upon differentiation, PRC2 is lost, enhancers acquire an active state (marked by H3K27ac), and the target genes are transcribed. Below: In the absence of PRC2 (PRC2 KO), poised enhancers lose H3K27me3 and do not contact their target genes in ESCs. Upon differentiation the enhancers fail to activate and do not acquire H3K27ac, and target genes are not transcribed. It is unclear whether RNAPII is also lost from promoters in these conditions.

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