Previews

The winding road toward transcriptional repression

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Enhancers are known for their role in mediating transcriptional activation. In this issue, Vermunt et al.[1] report the unexpected finding that genes can undergo a sequential transition between distinct enhancers to mediate progressive downregulation of expression.

Cellular differentiation is governed by complex transcriptional mechanisms that culminate in the progressive activation of cell type-specific genes and the simultaneous repression of genes from alternate lineages. Over the past few decades, considerable progress has been made toward our understanding of transcriptional regulation, which has been driven largely by discoveries related to the function of enhancers. In this issue, Vermut et al.[1] uncover a new twist in the function of enhancers by showing that a promoter can transition between different enhancers to mediate progressive transcriptional silencing.

Functionally defined as DNA elements that activate transcription of a gene in an orientation- and distance-independent manner,[2] enhancers possess a number of characteristics that reflect their function and facilitate their identification, including enrichment in specific histone marks (e.g., histone H3K27 acetylation), the presence of non-coding enhancer RNA (eRNA), and three-dimensional proximity with the promoter(s) of the gene(s) they activate. Importantly, enhancers provide an exquisitely refined level of transcriptional regulation, effectively controlling the temporal and spatial expression of genes during differentiation and development.[3] Furthermore, enhancers determine the amounts of transcripts being synthesized by regulating transcriptional bursting frequency.[4,5]

While the control of gene activation by enhancers during cell differentiation has been extensively characterized, we know very little about the progressive silencing of genes as progenitors progress along the developmental path to becoming specialized cells. To address this question, Vermunt et al.[1] took advantage of a highly synchronous cell differentiation system based on the inducible activation of the master regulator of erythroid differentiation GATA1. In this system, GATA1 is fused to the ligand-binding domain of the estrogen receptor (GATA1-ER) and, as such, remains in the cytoplasm of erythroid precursors. Upon estradiol treatment, GATA1-ER translocates to the nucleus and initiates a cascade of changes in gene expression that recapitulate terminal erythroid maturation. At first, Vermunt et al.[1] focused on the well-characterized gene *Kit* that is progressively silenced during erythroid maturation. Examining a potent enhancer located 114 kb upstream of the *Kit* transcription start site (−114), they detected a progressive loss of the active histone H3K27 acetyl mark (H3K27ac), a result consistent with transcriptional repression. Surprisingly, they also observed a corresponding increase in H3K27ac at several intronic regions suggesting the formation of new enhancers while the gene undergoes downregulation. Consistent with this, nascent non-coding transcripts that correspond to eRNA were also detected at these sites concomitantly with the increase in H3K27ac. Furthermore, 4C-seq assays confirmed the formation of *de novo* loops between the *Kit* promoter and these intronic sites. Taken together, these results reveal novel enhancer-like elements that appear at intronic sites while the *Kit* gene is being progressively silenced during terminal erythroid differentiation. Interestingly, these intronic enhancer-like elements are transient: they appear shortly after the beginning of *Kit* downregulation, peak 13 h after induction of differentiation, and disappear as the gene completely switches off.[1] While it is well established that the same promoter can be activated by multiple enhancers acting additively, synergistically, or redundantly (i.e., shadow enhancers),[6,7] the changes in enhancers that occur during silencing of the *Kit* gene are highly unusual in their kinetics, with decommissioning of the upstream enhancer (−114) occurring first, followed by activation of intronic enhancer-like elements that are themselves turned off later when *Kit* becomes completely repressed.

What could be the role of these transient enhancer-like elements in repressing the *Kit* gene? The answer to this question came from CRISPR/Cas9 deletion of the +49kb intronic element, which led to loss of contact with the promoter and accelerated repression of *Kit*. This effectively shows that the element works as a transient activator that counteracts *Kit* repression during erythroid differentiation. Further confirming the role of the +49 site as an activator of *Kit*, deletion of the −114 enhancer led to variegated *Kit* expression in the presence but not the absence of the +49 site. Furthermore, spatial proximity between the +49 site and the *Kit* promoter was maintained in cells that express residual levels of *Kit* (10%) but not in cells with a complete loss of *Kit* expression.[1] Overall, these results reveal that repression of the *Kit* gene during erythroid maturation does not follow a linear path, but rather entails a series of steps that include enhancer decommissioning but also paradoxically the activation of transient enhancers that slow down *Kit* repression, presumably to
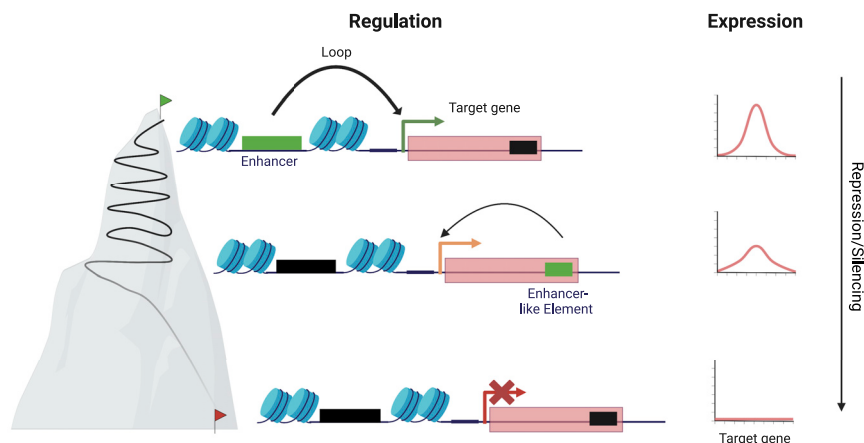
Figure 1. An illustration of the mechanisms that gradually repress transcription via formation of an enhancer-like element within intronic regions of the targeted gene
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prolong cell proliferation of erythroid precursors (Figure 1). Importantly, such transient enhancer-like elements are not limited to *Kit* but have been observed at hundreds of genes that undergo progressive silencing in both primary erythroid cells and adipocytes, suggesting this is a widespread phenomenon. Thus, while enhancers were previously known to act additively, synergistically, or redundantly,[6,7] this study[1] provides an important new twist to these functions by showing that enhancers can also act sequentially to mediate progressive downregulation of gene expression.

While a controlled, gradual decrease in transcription is understandably important for cell differentiation, one may wonder about the advantage of a mechanism that entails transitioning from a strong to a weak enhancer rather than simply decreasing enhancer strength through cofactor exchange and/or decreased transcription factor (TF) binding via post-translational modifications. Part of the answer may lie in the previous finding that the DNA sequence underlying TF binding motifs is critically important for enhancer activity with many enhancers containing DNA motifs that are suboptimal for TF binding.[8] While it is possible to create very strong enhancers by optimizing DNA motifs (or the spacing between DNA motifs) for increased TF affinity, these enhancers lose spatial and

temporal regulation,[8] suggesting that strong enhancers are difficult to regulate quantitatively. This may become an issue for genes that are controlled by very strong enhancers but must be progressively downregulated rather than abruptly turned off. In this context, the use of a relatively weaker enhancer that transiently takes over activation of a gene after the decommissioning of a stronger enhancer provides an effective solution for a controlled, gradual decrease in transcription.

An important unresolved question is whether the newly identified enhancers that serve as intermediates in transcriptional repression represent weaker versions of enhancers or whether they differ fundamentally in other properties of the *cis*-regulatory code, such as TF motif organization, genomic position (e.g., intra- or intergenic), or their capacity to recruit specific cofactors.[9] A recent study showed that distinct types of enhancers display different dependencies toward cofactors.[10] In this context, it is interesting to note that silencing of the transient *Kit* enhancer required a GATA1 protein capable of binding to the corepressor complex Nucleosome Remodeling and Deacetylase (NuRD), suggesting that the recruitment of corepressors (along with coactivators) may be characteristic of these transient enhancers. Much remains to be learned about the cofactor dy-

namics that occur at genes as they are becoming progressively silenced.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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