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Preview

Distance matters: How protein regulators facilitate enhancer-promoter interactions and transcription

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Cohesin, transcription factors (TFs), and mediator complex components regulate gene expression partly by regulating enhancer-promoter (E-P) communication. A new study combined E-P distance-controlled reporter screens with the inhibition or degradation of regulatory proteins and uncovered a distance-dependent effect across cohesin, TFs, and mediator complex components.

Enhancers are the key units of transcriptional regulation in mammals and ensure cell-type-specific gene expression. Dysregulation of enhancers is a major cause of diseases and developmental defects.[1] Cognate enhancers and promoters can be located far apart in the linear genome—sometimes separated by more than a megabase—yet, enhancers loop to their target genes to activate them. This raises the question of how enhancers find their target promoters and how this process is regulated. Developments in the 3D genome field over the past decade have revealed that 3D chromatin organization appears to largely result from two mechanisms: (1) affinity-based compartment-type interaction including canonical larger-scale A/B compartments and finer-scale microcompartments and (2) loop extrusion-based chromatin interactions that result in the formation of topologically associating domains (TADs) and loops.[2,3] In particular, TADs have been considered primary boundaries that limit enhancer-promoter (E-P) communication, although they are dynamic over time.[4] Loop extrusion by cohesin and stalling at CTCF sites can explain the formation of TADs and loops.[5] E-P interactions often span tens to hundreds of kilobases, which raises the question of how loop extrusion facilitates and/or interferes with E-P communication. In recent years, numerous studies have aimed to address this question but have yielded various seemingly contradictory conclusions. While some studies utilizing local manipulation of CTCF sites or loop extrusion have reported strong effects on gene expression, other studies using global disruption of cohesin or CTCF show that the majority of genes are insensitive to these changes.[3] Thus, the relationship between E-P communication and loop extrusion in enhancer biology still remains unresolved.

In this issue of *Cell Genomics*, Tjalsma et al. leveraged distance-controlled reporter screens in K562 cell lines to investigate the relationship between E-P distance and the effects of specific protein repression.[6] They previously generated cell lines capable of monitoring GFP expression driven by the *HBB*-like *HBG1* promoter regulated by the locus control region (μLCR), a compact version of the *HBB* super enhancer, which was integrated at two distances (0 and 100 kb) from the reporter gene (E0 and E100).[7] Tjalsma et al. also generated two new cell lines. One, EC100, incorporates three strong CTCF binding sites immediately downstream of the enhancer region and oriented toward the enhancer region, and the other, E−50, has μLCR positioned at −50 kb from the reporter gene, allowing analysis of the effects of CTCF and enhancer orientation (Figure 1A). They performed a CRISPRi screen using ∼18,000 sgRNAs targeting 3,200 nuclear proteins (Figure 1A). First, they found that the repression of four cohesin complex subunits (SMC1A, SMC3, RAD21, and STAG2) had a profound negative impact on long-range enhancer activation in E−50, E100, and EC100. Interestingly, repression of cohesin complex components had the opposite effect in the E0 line, in which it increased expression. Second, Tjalsma et al. observed that eight components of the mediator complex, including MED14, also exhibited a distance-dependent bias, showing a more pronounced negative effect on long-range controlled reporters. However, inhibition of these mediator complex components generally resulted in the repression of GFP reporter expression even in E0. Finally, the authors demonstrated that the knockdown of tissue-specific transcription factors (TFs), including LDB1, led to the downregulation of the reporter gene without a clear bias toward E-P distance.

To further investigate distance-dependent effects of the cohesin complex, they generated a RAD21 degron cell line, in which RAD21 can be rapidly degraded (Figure 1A). They performed nascent RNA sequencing (BrU-seq) with and without RAD21 degradation to analyze which genes exhibited expression changes. As partly expected from the CRISPRi screen, at the endogenous *HBB* locus, *HBG1* and *HBG2*, which are regulated by the 20-kb- and 25-kb-distal LCR enhancer, were downregulated, while HBE1, regulated by the 2.5-kb-proximal LCR enhancer, was upregulated. Furthermore, to analyze

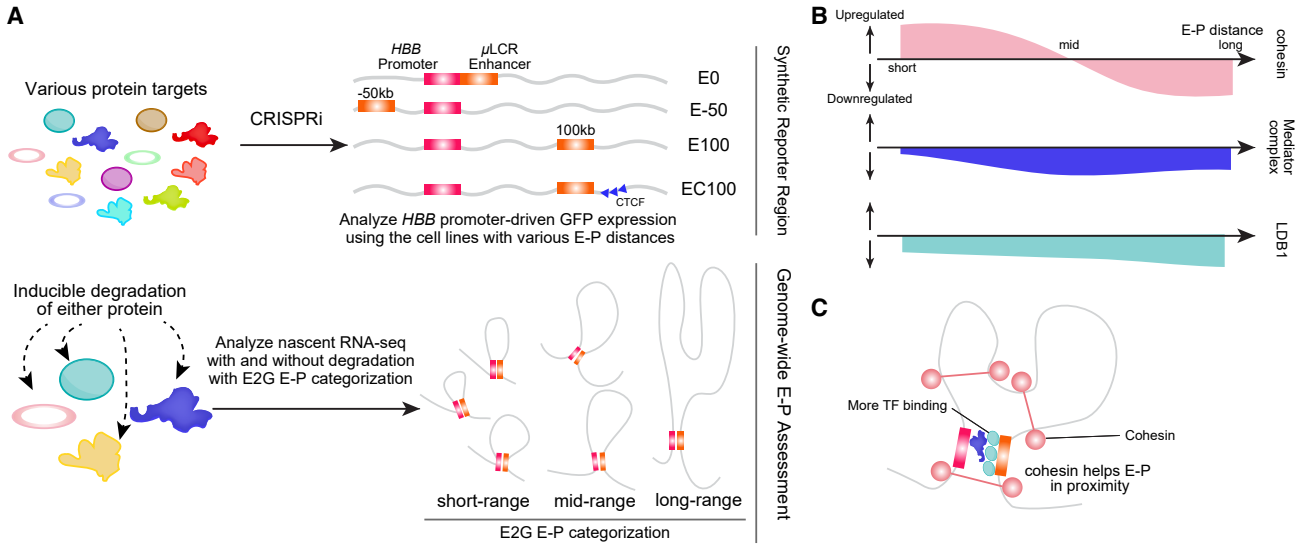

Figure 1. Systematic inhibition or degradation of regulatory proteins reveals the distance-dependent effects on E-P gene regulation

(A) Top: following CRISPRi screening, the effects on GFP expression driven by the *HBB* promoter, regulated by μLCR at various distances from the promoter region, were analyzed. Bottom: nascent RNA-seq was performed with and without targeted protein degradation of a specific protein to compare gene expression changes. Genome-wide E-P pairs, identified based on the ENCODE-rE2G model, were categorized to clarify distance-dependent effects.

(B) Effect on gene expression under cohesin, mediator complex, and LDB1 degradation in relation to E-P distance. Cohesin degradation leads to the upregulation of short-range controlled genes and the downregulation of long-range controlled genes. In contrast, the degradation of mediator complex components and TFs leads to gene downregulation across all three categories, with progressively stronger effects on mid- and long-range E-P pairs, respectively.

(C) Putative model of interplay among regulatory proteins. In long-range E-P pairs, cohesin facilitates E-P proximity, likely through the loop extrusion process. Mediator complex components and an increased number of bound TFs also contribute to E-P-mediated transcription.

genome-wide gene expression changes in relation to E-P distance, Tjalsma et al. adopted the ENCODE-rE2G model and categorized promoters into four groups: promoter autonomously, short range (2–10 kb), mid-range (10–40 kb), and long range (50–500 kb), based on the presence and distance of enhancers (Figure 1A). By analyzing these categories, they observed that enhancer-dependent genes become more tissue specific as E-P distance increases, whereas approximately half of promoter-autonomously expressed genes are ubiquitously expressed. They also found that long-range controlled genes had weaker promoter features and were associated with stronger enhancers. Through the analysis of genes affected by RAD21 depletion, they found that long-range controlled genes were significantly downregulated, while short-range genes were upregulated, consistent with their observations in the CRISPRi screening (Figure 1B). When they analyzed enhancer strength, represented by EP300 levels in long-range E-P genes, they found that RAD21-depletion-responsive genes had higher EP300 levels than non-responsive genes, suggesting that enhancer strength is an important factor for long-range E-P activation. However, even after stratifying by enhancer strength, short-range E-P genes remained insensitive to RAD21 depletion, indicating that E-P distance, rather than enhancer strength, is the key factor for cohesin-depletion sensitivity.

They also investigated whether mediator complex components or TFs exhibit a similar biased effect on long-range controlled genes using the inducible degron system combined with nascent RNA-seq (Figure 1A). Their results demonstrated that mid- and long-range controlled genes were significantly sensitive to MED14 degradation, whereas short-range controlled genes were not (Figure 1B). Next, they tested the LDB1-FKBP degron cell line. While they did not observe a distance bias for TFs in the CRISPRi screening experiment, by performing a genome-wide assessment of LDB1 knockdown, they found that long-range and mid-range controlled genes were more sensitive to LDB1 depletion (Figure 1B). Additionally, LDB1 enrichment on enhancers correlated well with the response to LDB1 depletion, suggesting that LDB1 is more frequently recruited to distal enhancers, where LDB1 may affect gene expression by regulating enhancer strength and/or E-P loop strength. In addition, the loss of LDB1 specifically reduced E-P interactions, consistent with a recent report.[8]

To further expand their observations, they conducted CRISPRi experiments targeting each factor, followed by RNA-seq. While the depletion of factors involved in basal transcription, such as SUPT5H or POLR2H, downregulated many genes without any clear bias toward a specific gene category, many cohesin-related and mediator-related factors had a highly significant impact on long-range controlled genes. Interestingly, enhancer biology-related factors also had a pronounced impact on long-range E-P genes. Collectively, their results demonstrated that long-range E-P-controlled genes were highly sensitive to protein-level perturbations—not only cohesin but also many factors recruited to enhancers to support their activity.

Finally, they validated their findings using a public dataset involving the chemical inhibitor JQ1, a BET bromodomain inhibitor implicated in (super)enhancer regulation and general transcription. While a high dose globally repressed transcription, mid- and long-range controlled genes

were more sensitive to a low level of JQ1 treatment.

To put the results from Tjalsma et al. into context, it is worth considering the several mechanisms that bring distal enhancers and promoters together,[1] which include (1) passive 3D diffusion; (2) active loop extrusion without CTCF sites at enhancers and promoters; (3) loop extrusion with facilitating CTCF sites at the enhancer and/or promoter; and (4) additional but less well-understood mechanisms such as specific looping factors like LDB1. These mechanisms may simultaneously operate and are not mutually exclusive, but each mechanism is likely to show distinct sensitivity to the loss of a specific protein regulator as well as distinct distance dependence.[1] Indeed, in this study, Tjalsma et al. meticulously demonstrated that cohesin, mediator complex components, and TFs regulate transcription through E-P interactions in a distance-biased manner. Regarding the mechanisms underlying this distance dependency, while greater enrichment of TFs at long-range enhancers may contribute to distal E-P regulation, cohesin appears to be particularly important at long E-P distances (Figure 1C). This raises a key question for future research: how exactly does loop extrusion bring E-P pairs into spatial proximity for facilitating their transcription? Live-cell imaging of E-P interactions using DNA locus labeling,[4] ideally combined with nascent transcription analysis, could help answer these questions. Furthermore, since the cohesin variants STAG1-cohesin and STAG2-cohesin play differential roles in loop regulation,[9] it would be interesting to investigate how these cohesin variants, with differing loop extrusion capacities, differentially regulate E-P interaction-mediated transcription in this system. Another intriguing finding is that both MAU2 and WAPL knockdown resulted in significant downregulation of long-range controlled genes, despite their knockdown presumably leading to opposite effects on loop extrusion processivity. While several explanations are possible, one potential hypothesis is that if cognate E-P pairs may have evolved to match a certain cohesin processivity resulting in an "optimal range for E-P pair," such that any defect, whether increasing or decreasing loop extrusion processivity, may alter this range and result in transcriptional dysregulation. In MAU2 knockdown, the reduced processivity or extrusion would be expected to result in shorter or fewer loops, which may fail to bring promoters and enhancers into proximity, disrupting long-range E-P interactions. Conversely, a previous report suggests that WAPL knockdown causes redistribution of cohesin, which depletes E-P loops accompanied by downregulation of target genes.[10] This may also ultimately lead to the downregulation of long-range controlled genes. Overall, Tjalsma et al. provide extensive new insights into how different proteins regulate E-P interactions and gene regulations, thus moving the field significantly forward.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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