Enhancer–promoter interactions and transcription

A new study addresses whether transcription of enhancers and the resulting enhancer RNAs (eRNAs) play a role in mediating long-range interactions between enhancers and promoters. Studying the immunoglobulin heavy chain (Igh) locus, the authors find that transcription of the enhancers per se is required to establish but not maintain these interactions, and this mechanism may apply to a subset of other enhancer–promoter interactions.

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Over the past 20 years, broadly, three classes of fundamental regulatory elements controlling gene expression have emerged: enhancers, promoters and boundary elements. These elements switch genes on or off accurately in time and space in response to intrinsic and external signals. Such elements controlling the expression of even a single gene are now known to be distributed throughout very large segments of the genome1. Current research focuses on how these fundamental elements interact in controlling gene expression, particularly how activated enhancers communicate and transfer integrated information to their cognate promoters. Enhancers, when activated, in many cases appear to make physical contact with the promoters that they control, although there are exceptions1. This finding has led to a long-standing question of how enhancers, which are often located far (tens to hundreds of kilobases) from promoters, make contact in the three-dimensional context of the nucleus in real time. Since the first observations demonstrating the need for long-range enhancer–promoter interactions in the globin loci2,3, three overlapping hypotheses have been proposed involving chromatin looping, linking via polymerized proteins and tracking of molecular motors along chromatin (reviewed in ref. 1). Currently, the most popular model includes tracking driven by the cohesin complex, which results in directed rather than random looping4. However, the story is incomplete, because acute removal of cohesin does not cause the perturbation of gene expression that might be predicted if cohesin-driven interactions were the only mechanism mediating enhancer–promoter interactions5. Another somewhat controversial candidate for mediating enhancer–promoter interactions, discussed in this issue of Nature Genetics, is transcription via RNA polymerase II (Pol II). Activated enhancers are complex, dynamic multiprotein structures6 characterized by a small (200–600 base pair) nucleosome-free stretch of DNA bound by tissue- and developmental-stage-specific transcription factors and cofactors. Included in the mix of associated proteins are the multisubunit cohesin complex and the Mediator complex. The flanking nucleosomes are typically modified by histone acetylation (for example, acetylation of histone H3 K27), and importantly most activated enhancers are bound by Pol II and transcribed in both directions, thus prompting comparisons between enhancers and promoters7. Finally, when transcribed, the chromatin associated with enhancers is increasingly modified (typically by mono- or dimethylated H3 K4) by MLL–COMPASS complexes. The dynamic mechanisms linking promoters and enhancers are further complicated by the finding that during each cell cycle, these long-range interactions are likely to be disassembled and must be remade8. Experiments altering the transcription of enhancers are difficult to design, because altering the signals required for transcription might also affect other aspects of enhancer activity in unpredictable ways. Now Fitz et al.9 have used a different approach by altering transcription in general by downregulating Spt5, a highly conserved transcriptional regulator that modulates Pol II pausing and processivity. In a genome-wide analysis, they found a substantial number of enhancer– promoter pairs whose expression was coordinately downregulated when Spt5 was decreased, and they concentrated their detailed analysis on one such pair at the mouse immunoglobulin heavy chain (Igh) locus. Expression of germline transcripts from all Igh constant- and variable-region promoters is controlled by a group of four redundant distal

enhancers (a so-called super-enhancer). During B-cell development, these enhancers acquire the characteristic signatures of activated enhancers and physically contact the relevant Igh promoters. Deletion of all four enhancers abrogates expression from the Igh promoters. Fitz et al.9 show that the phenotype of B cells depleted in Spt5 appears identical to that of cells in which the Igh super-enhancer has been physically deleted, thus suggesting that decreasing transcription of the enhancers in some way abolishes their activity. The authors rule out an effect of Spt5 depletion on the Igh genes themselves. In a series of very well controlled experiments, the authors go on to show that, other than the lack of transcription, in all other respects the enhancers appear to be charged with all the proteins and chromatin modifications that typify the fully activated Igh enhancers (Fig. 1). However, unexpectedly, the authors found that, in the absence of transcription, the fully activated enhancers no longer physically engage the Igh promoters even though they still bind components (Rad21) of the proposed tracking-protein complex (cohesin) (Fig. 1). Importantly, reinstating transcription of the enhancers by using strong activators linked to a catalytically inactive Cas9, looping and transcription of the promoters is restored. In a final series of experiments, the authors ask which aspect of the enhancer– promoter interaction requires transcription. By inhibiting either elongation (with flavopiridol treatment) or initiation (with triptolide treatment), the authors found that, in general, transcription of the enhancer is required to establish the enhancer–promoter interaction but not to maintain it. Given that this interaction must be at least partially disrupted every time the cell replicates its DNA and divides, transcription of the enhancer will be required at some point during each cell cycle to re-create the interaction. Why transcription might influence enhancer– promoter interaction is unknown. The authors speculate that the energy released by Pol II translocation might lead to non-thermal molecular agitation or 'stirring', thus conferring greater mobility to transcribed enhancers. This enhanced mobility might allow for faster and more efficient sampling of the surrounding nuclear space10. This finding harks back to the model of random looping. Although this model is plausible, others have found the opposite: that transcription restricts the movement of chromatin11. Altogether, these findings, set in the context of many conflicting observations on the relationship between transcription, eRNAs and enhancer–promoter interactions, suggests that evolution has solved the problem of achieving enhancer–promoter communication in many diverse ways, some of which involve the transcription of enhancers. ❐

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Competing interests

The author declares no competing interests.