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Review

Enhancer Function: Mechanistic and Genome-Wide Insights Come Together

Jennifer L. Plank[1] and Ann Dean[1,*]
[1]Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: anndean@helix.nih.gov
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Enhancers establish spatial or temporal patterns of gene expression that are critical for development, yet our understanding of how these DNA *cis*-regulatory elements function from a distance to increase transcription of their target genes and shape the cellular transcriptome has been gleaned primarily from studies of individual genes or gene families. High-throughput sequencing studies place enhancer-gene interactions within the 3D context of chromosome folding, inviting a new look at enhancer function and stimulating provocative new questions. Here, we integrate these whole-genome studies with recent mechanistic studies to illuminate how enhancers physically interact with target genes, how enhancer activity is regulated during development, and the role of noncoding RNAs transcribed from enhancers in their function.

Introduction

Enhancers are *cis*-acting DNA regulatory elements that increase the transcriptional output of target genes to influence the destiny of cells during development and differentiation. Enhancers may reside far from their in vivo targets, raising key mechanistic questions about how they communicate with promoters. Models such as enhancer looping to distant targets, linking by large protein complexes, or tracking along intervening chromatin were animatedly discussed but failed to be definitely resolved (Bulger and Groudine, 2011). The development of chromosome conformation capture (3C) technology (Dekker et al., 2002) allowed physical interaction frequencies between specific enhancers and target genes to be determined (Tolhuis et al., 2002). This significant advance affirmed that enhancers establish proximity with the genes they activate, although the original models are not mutually exclusive and may yet be found to contribute to enhancer function.

Enhancer DNA sequences are replete with clusters of binding sites for transcription factors whose occupancy confers upon them tissue specificity. Thus, it had long been proposed that factors binding to enhancers and genes could stabilize chromatin loops between them through homotypic or heterotypic interaction. Indeed, tissue-specific proteins that are critical for looping have been identified in a limited number of mammalian model systems such as the β-globin locus and the human *IFNγ* and T_H2 cytokine loci, among others (Krivega and Dean, 2012). In addition, looping interactions of chromosome-organizing proteins, such as the insulator binding protein CTCF and its frequent partner cohesin, have direct and indirect roles in facilitating enhancer-gene chromatin contacts (Ong and Corces, 2014). Furthermore, lineage-specific activators and CTCF/cohesin engage in interactions with components of the RNA polymerase II (Pol II) machinery and with the Mediator transcriptional coactivator complex to tie enhancer loops directly to the transcription apparatus (Maston et al., 2012).

Whole-genome studies now place enhancer interactions in a 3D nuclear context. These advances have depended on refinements in 3C-related approaches, including 5C, Hi-C, and ChIA-PET, capable of detecting chromatin loops at multiple levels (de Laat and Dekker, 2012). Studies across developmental stages detail the dynamic regulation of enhancers and enhancer looping during development (de Laat and Duboule, 2013). Moreover, recent results suggest a function for enhancer transcription into eRNAs as part of the mechanism of gene activation and possibly looping (Ørom and Shiekhattar, 2013). The new data, coming in a deluge of publications over a very recent period, paint a picture of multiple levels of long-range genome interactions, of which enhancer-gene contacts are a part. Descriptive by nature, the studies nevertheless allow mechanistic insights and raise new questions such as how chromosome folding occurs, what drives the changes in enhancers during development, and how enhancer looping and transcription activation are integrated physically and spatially in the nucleus to achieve a unique gene expression pattern.

Excellent recent reviews have covered genome-wide discovery of new enhancers and gene targets, activator and coactivator recruitment to enhancers, and specific features of enhancers such as an open chromatin structure, the H3K4me1 histone mark, and histone acetyltransferase p300 occupancy (Spitz and Furlong, 2012; Maston et al., 2012; Calo and Wysocka, 2013). Here, we specifically focus on mechanistic insights into enhancer function from individual gene and very recent genome-wide studies. We begin by considering the basis for enhancer-gene loops in individual loci and across chromosomes. We then discuss how enhancer activity is regulated during development to modulate gene expression. We end with an account of our current understanding of enhancer RNAs and their roles in enhancer activity and gene regulation.

Enhancers Engage in Long-Range Interactions with Target Genes through Lineage-Specific Factors and Ubiquitous Architectural Proteins

Enhancers are typically occupied by clusters of transcription factors that exclude nucleosomes and contribute to their DNase I

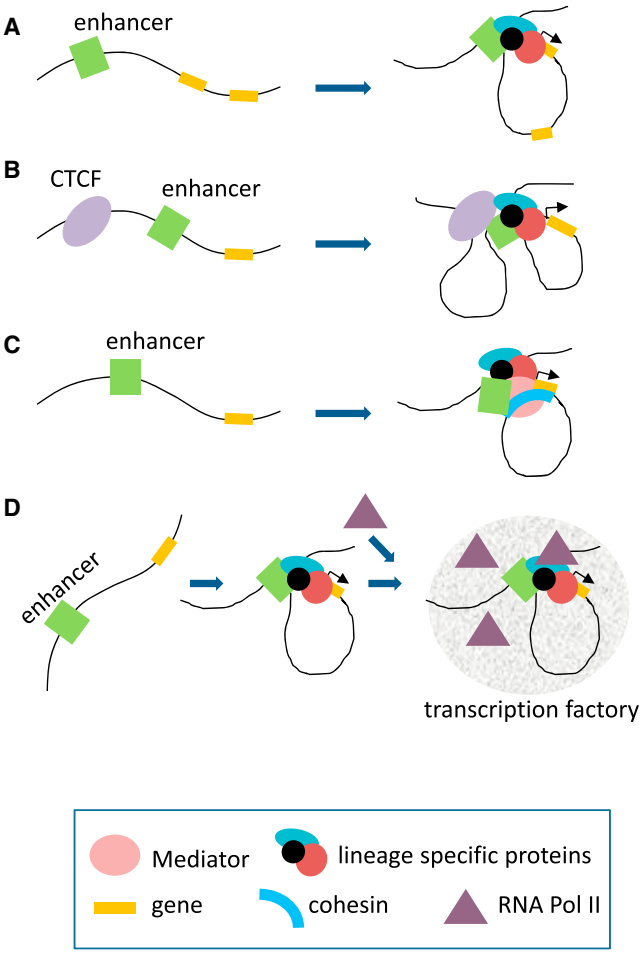

Figure 1. Enhancers and Promoters Communicate by Chromatin Looping

(A) Left: Two lineage-specific genes and an enhancer are depicted along unfolded chromatin with neither gene being transcribed. Right: Lineage-specific transcription factors mediate long-range interaction between the enhancer and one of the genes through homotypic and/or heterotypic protein interaction. The gene in contact with the enhancer is activated; the other gene (inactive) is looped away from the elements that are in proximity.

(B) Left: A CTCF binding site and an enhancer are depicted with an inactive gene along unfolded chromatin. Right: The gene is activated by lineage-specific activators that co-opt CTCF into long-range interaction with the gene.

(C) Left: A noninteracting enhancer and gene. Right: The enhancer is bridged to the gene promoter by Mediator and cohesin with participation of lineage-specific factors, activating the gene.

(D) Left: A locus containing a gene and enhancer reside in an unfolded and inactive state. Center and right: Enhancer-gene looping is depicted as being mediated by lineage-specific activators before accumulation of Pol II and the appearance of a transcription factory and transcription.

hypersensitive character. At select loci, 3C and RNAi studies have shown that specific enhancer binding proteins are required for enhancer-gene looping (Figure 1A). For example, a complex including GATA1 and cofactor FOG1 along with TAL1, LMO2, and LDB1 is required for β-globin locus control region (LCR) looping to globin genes and for transcription activation in mature erythroid cells (Vakoc et al., 2005; Song et al., 2007; Yun et al., 2014). The dimerization domain of LDB1 underlies the enhancer-gene proximity (Krivega et al., 2014). In fact, the dimerization domain alone, when linked to the β-globin promoter via a specially designed DNA binding zinc finger protein, is capable of driving loop formation and partially activating transcription in immature erythroid cells where this does not normally occur, arguing for the causality of the loop in the activation (Deng et al., 2012). A large cohort of erythroid genes is regulated by the LDB1 complex, suggesting it can function broadly, likely through chromatin looping, to affect lineage commitment (Li et al., 2013a).

Other focused investigations in the *IFNγ* and *MYB* loci indicate that architectural proteins CTCF/cohesin can participate directly in enhancer-gene looping (Figure 1B) (Sekimata et al., 2009; Stadhouders et al., 2012; Hadjur et al., 2009). For example, enhancers that bind the lineage-specific factor T-BET in T_H1 cells are interspersed with CTCF/cohesin binding elements in the *IFNγ* locus (Sekimata et al., 2009). As naive T cells differentiate, CTCF promotes a T_H1 cell-specific *IFNγ* locus looped conformation joining both kinds of sites and activating *IFNγ* transcription. CTCF occupancy and looping are dependent on enhancer binding by T-BET, but whether these proteins interact is unknown. These examples illustrate not only how lineage-specific transcription activators mediate enhancer-gene looping but also how they can cooperate with a ubiquitous looping factor (CTCF) to drive a cell-type-specific regional architecture conducive to transcription.

Enhancer-gene interactions are dismantled during cell division. Interestingly, recent studies indicate that key lineage factors FOXA1 in hepatoma cells and GATA1 in erythroid cells remain associated with select sites on mitotic chromosomes, some of which have enhancer markings (Caravaca et al., 2013; Kadauke et al., 2012). Moreover, cohesin remains on chromatin during mitosis in colon cancer cells after eviction of clustered transcription factors with which it was associated at enhancer-like sites in interphase (Yan et al., 2013). We speculate that enhancers may be appropriate repositories for mitotic bookmarks to re-establish these long-range interactions pattern after cell division.

Enhancer Loops Are Tied to the RNA Polymerase II Transcription Complex and Transcription Activation

The enhancer's main job is to increase transcriptional output. This activity could be manifest at different stages, including transcript initiation, elongation, or termination. Enhancer-promoter interactions can involve components of the basal transcription machinery (Ren et al., 2011; Liu et al., 2011; Koch et al., 2011). Moreover, Mediator occupies enhancers of many ES cell genes together with pluripotency factors OCT4, SOX2, and NANOG (Kagey et al., 2010) and bridges the enhancers to Pol II at target promoters by direct interaction with cohesin (Figure 1C). These studies intimately link enhancer loops to transcription initiation. Other studies suggest a role for enhancers in elongation (Sawado et al., 2003; Deng et al., 2012). New work now shows that certain enhancers specifically function to release Pol II pausing and allow elongation (Liu et al., 2013). These anti-pause enhancers loop to target promoters and permit activation of the P-TEFb complex, which is required for release of Pol II into elongation. Enhancers, thus, have diverse mechanistic functions in transcriptional regulation.