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Enhancer RNAs: the new molecules of transcription

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Abstract

In the past few years, technological advances in nucleotide sequencing have culminated in a greater understanding of the complexity of the human transcriptome. Notably, the discovery that distal regulatory elements known as enhancers are transcribed and such enhancer-derived transcripts (eRNAs) serve a critical function in transcriptional activation has added a new dimension to transcriptional regulation. Here we review recent insights into the tissue-specific and temporal-specific gene regulation brought about by the discovery of eRNAs.

Introduction

Genome-wide analysis of transcription factor binding sites has revealed a majority of their chromatin residence at distal intragenic and intergenic regions that exhibit features associated with enhancers [1–6]. There is a general consensus that enhancers govern tissue-specific and temporal-specific regulation of gene expression [7–9]. These findings are in line with the importance of enhancers in signal-dependent transcriptional responses and the evolutionary conservation of enhancer elements. Mapping co-activator binding sites and unique chromatin modification signatures at potential enhancer-like sequences provided further insight into the characterization of enhancers. It was found that most active enhancers are not only marked by p300/CBP binding but also contain histone H3 monomethyl lysine 4 and H3 lysine27 acetylation modifications [3,10,11]. The exact function of such histone modifications at enhancers is not known. However, recent biochemical and genetic studies have identified the MLL3/MLL4-containing complexes in deposition of monomethyl H3 lysine 4 at enhancers [12]. It is likely that such enhancer-associated histone marks are serving as a signal or a platform for important enhancer-binding factors or activities, as was shown for HP1 binding to H3 lysine 9 methylation marks at heterochromatic regions of the genome [13]. Importantly, since enhancers are critical regulatory regions during cellular differentiation, their modulation during disease progression could be of utmost relevance. Recent experiments have revealed that many silenced embryonic stem cell DNase I-hypersensitive sites (DHSs), which are a general feature of enhancers, are reactivated in cancer [14]. Surprisingly, many of the reactivated DHSs corresponded to sites distinct from those that were active in cell lineages from which the malignancy was derived [14]. Collectively, these results point to enhancers as critical elements whose reactivation may underlie disease-inducing gene expression programs.

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Transcription at enhancers

While enhancers were known to bind sequence-specific transcription factors, the association of RNA polymerase II (RNAPII) and general transcription factors with enhancers came as a surprise [15••,16•]. Further experiments indicated that enhancers are transcribed as long noncoding RNAs and such transcripts displayed stimulus-dependent activation [15••,17••]. Importantly, a number of experiments pointed to a strong correlation between the transcription of enhancers and increased expression of their neighboring genes, suggesting a functional relevance for enhancer-mediated transcription [15••,17••]. The precise nature of enhancer-derived transcripts (named eRNAs) has not been defined. While transcriptional co-activator CBP and RNAPII are centered at sequence-specific transcription factor sites, eRNAs are transcribed bi-directionally emanating from the RNAPII peaks. Interestingly, while a large number of enhancers are transcribed bi-directionally, there is a group of enhancers that are induced uni-directionally [18,19•]. Currently, it remains unclear why some enhancers are transcribed from both strands and others are unidirectional. It is likely that the nature and number of transcription factor binding sites at a given enhancer determine the directionality of transcription. Moreover, additional regulatory co-factors may cooperate with RNAPII to govern the directionality of eRNAs.

Of critical importance is the mechanism by which the primary transcripts of eRNAs proceed to their mature forms. While the majority of eRNAs are reported to be monoexonic and not polyadenylated, there have been cases where eRNAs are spliced and polyadenylated [15••, 16•,17••]. Moreover, it is unknown whether a single enhancer is transcribed as a mixture of polyadenylated and non-polyadenylated eRNAs or if different classes of enhancers express one or the other form of the transcript. However, there is a general agreement that the majority of the reported eRNAs correspond to transcripts of about 2–5 kilobases (kb) in length. While eRNAs were originally detected using high-throughput RNA sequencing of steady state levels of total RNAs, it is becoming clear that techniques such as Global nuclear run-on sequencing (Gro-Seq), which measures the pioneering rounds of transcription, are more suitable for the detection and quantitation of eRNA changes [18,19•,20]. This may be the result of the general instability of eRNAs compared to that of messenger RNAs, consistent with the observation that eRNAs are predominantly not polyadenylated.

Functionality of enhancer RNAs

Initial reports correlating the transcription of eRNAs and their neighboring protein-coding genes were suggestive of the functional relevance of eRNAs in transcriptional activation. However, the exact requirements for eRNAs in activation of their target genes were not determined. Two reports provided evidence linking the long noncoding RNAs to transcriptional activation. Using knockdown approaches, it was shown that long noncoding RNAs positively regulate their neighboring protein-coding genes [21••]. Analysis of the genomic sites for noncoding RNA (termed ncRNA-a3) that regulated the TAL1 gene (also known as SCL) revealed its intimate association with enhancers as defined by enrichment in monomethyl H3K4 and acetylated H3K27 marks [21••,22]. This locus produced bi-directional transcripts that were polyadenylated and spliced. A similar approach was used to show that a noncoding RNA termed HOTTIP activates several 5′ HOXA genes *in vivo* [23].

HOTTIP also corresponded to a spliced and polyadenylated transcript. While these experiments revealed a role for long noncoding RNAs in activation of neighboring protein-coding genes, they did not assess the specific signaling pathway(s) that is regulated by long noncoding RNAs.

Subsequent studies analyzing the activation of transcription through a number of different signaling pathways extended the role of eRNAs in enhancer function. Genome-wide studies revealed p53 association with a large number of distal binding sites [24•]. Detailed analysis of two distinct extragenic p53 sites revealed the expression of eRNAs that were stimulated following treatment of cells with Nutlin-3, an inducer of p53. Importantly, depletion of eRNAs corresponding to each enhancer abrogated the Nutlin-3 induction of transcription of the targeted protein-coding gene [24•]. A similar scenario was observed following activation of estrogen-responsive genes in MCF7 cells [19•]. Treatment of MCF7 cells with estradiol (E2) induced the binding of estrogen receptor alpha (ER-alpha) to a large number of extragenic binding sites. Majority of the E2-upregulated protein-coding genes contained an E2/ER-alpha-binding enhancer within 200 kb from their transcription start sites [19•]. Most of these E2-induced enhancers produced bi-directional eRNAs as measured by Gro-Seq. Critically, depletion of eRNAs corresponding to a number of enhancers diminished the E2-induced activation of their target genes. It was further shown that at least in the case of one enhancer involved in activation of FOXC1 gene, only the sense strand of eRNA contained the activating function. This suggests that perhaps only one strand of eRNAs confers transcriptional activation [19•].

Further evidence to the functionality of eRNAs was provided following studies of nuclear receptor Rev-Erbs in mouse macrophages [20]. It was found that Rev-Erbs predominantly bound extragenic and intragenic enhancer-like regions to repress their neighboring protein-coding genes. This was highlighted for two Rev-Erbs-responsive genes, Mmp9 and Cx3cr1. Evidence was found of bidirectional transcription corresponding to eRNAs from a majority of Rev-Erbs-associated extragenic enhancers. Detailed examination of two distinct sites adjacent to Mmp9 and Cx3cr1 genes revealed that Rev-Erbs binding at these enhancers resulted in repression of eRNAs expression, leading to silencing of the targeted genes. Moreover, depletion of eRNAs corresponding to each enhancer led to a specific repression of neighboring protein-coding genes, demonstrating the functional importance of eRNAs. Significantly, they show that only one strand of the eRNA is involved in the activating function, which begs the question of the functional importance of the other strand. It is important to note that most protein-coding genes also contain bidirectional transcription of unknown function [25]. Taken together, it is clear that most activity-dependent enhancers express eRNAs and in nearly all cases examined, such eRNAs are endowed with functional information required for transcriptional activation.

Enhancer RNAs mechanism of action

How distal regulatory elements confer transcriptional activation of their target genes has remained a puzzle in transcription. The prevailing idea put forward from recent advances in understanding the three-dimensional structure of the genome stipulates the formation of a DNA loop connecting the distal regulatory sites and the core promoter elements of the

protein-coding gene. In such a scenario sequence-specific DNA binding factors and their associated co-factors mediate the association of the enhancer and their targeted promoter sequences. Recent experiments have suggested that two multi-protein complexes, Mediator and Cohesin, play an important role in such stimulus-dependent chromatin looping [26,27]. More importantly, these experiments have suggested a role for eRNAs in either the establishment or the maintenance of enhancer–promoter contacts. Depletion of long noncoding RNAs involved in activation of SNAI1 or TAL1 resulted in decreased chromatin residence of Mediator at the promoter of these genes concomitant with diminished DNA looping as measured by chromosome conformation capture (3C) [22]. Similarly, knockdown of estrogen-induced eRNAs reduced the enhancer–promoter looping at a number of estrogen-responsive genes. The decrease in chromatin looping was accompanied with reduced levels of Cohesin occupancy at estrogen-induced enhancers [19•]. Collectively, these results paint a framework by which the Mediator and Cohesin complexes cooperate with the eRNAs to promote enhancer–promoter interactions (Figure 1).

While the augmentation of DNA looping serves as one possible mechanism by which eRNAs confer their responsiveness, there are other proposed mechanisms of action. The overall hypothesis envisions a role for eRNAs as a guide to recruit sequence-specific factors, chromatin remodeling or chromatin modifying complexes to the targeted promoters. Two recent studies proposed the association of activating long noncoding RNAs to transcription factors SOX2 or androgen receptor, resulting in increased chromatin residence of the transcription factors and activation of their specific gene expression programs [28,29]. In a different study, the long noncoding RNA HOTTIP was shown to recruit the MLL complex to the HOXA cluster resulting in its activation [23]. More recently, the eRNAs expressed from the core enhancer (CE) element of the MYOD gene were shown to increase RNAPII occupancy and chromatin accessibility at the MYOD promoter by the proposed recruitment of chromatin remodeling complexes [30]. Therefore, the overarching theme for these studies suggests the physical association of the eRNAs with a component of transcription regulatory machinery, resulting in increased chromatin association of a specific factor and enhancement of transcription. While it is likely that there may be common themes by which eRNAs expressed from different regulatory regions mediate their responsiveness, it would not be surprising to find specific eRNAs that utilize novel strategies to activate transcription.

Outlook and biological implications of enhancer RNAs

It is becoming clear that achieving a detailed molecular understanding of enhancers will require a greater insight into eRNA biogenesis, mechanism of action and regulation. Since enhancers control tissue-specific and temporal-specific gene expression, understanding the dynamic regulation of eRNAs during development and in disease progression will be of utmost importance. Recent studies have started to shed some light on the biological relevance of eRNAs. An enhancer-like RNA was shown to regulate neurogenin 1 expression during mouse cortical development [31]. The eRNA expressed from the CE element upstream of the MYOD1 locus was shown to regulate the MyoD expression during the differentiation of C2C12 cells, underscoring the importance of eRNAs in myogenic gene expression programs [30]. In a different study, it was shown that an eRNA expressed from p53-bound enhancer region 2 (p53BER2) activated the protein-coding gene PAPPA in

MCF7 cells [24•]. As expected, while treatment of MCF7 cells with infrared (IR) irradiation led to a G1 arrest, depletion of p53 or p21 could rescue such cell cycle arrest, confirming that the arrest is indeed p53 dependent. Importantly, depletion of PAPPA or the eRNA (derived from the p53BER2) regulating the PAPPA also had a significant inhibitory effect on the p53-induced cell-cycle arrest. The critical importance of these studies is the idea that targeting of specific eRNAs may be the key methodology that would allow for locus-specific, tissue-specific, and temporal-specific regulation of gene expression. It was possible to deplete the eRNAs controlling the Mmp9 gene leading to diminished Mmp9 expression *in vivo* [20] using lipofectamine-siRNA delivery following induction of sterile peritonitis in mice. These experiments provided the proof of principle that targeting of eRNAs to reduce mRNA expression could be a viable methodology to control locus-specific gene expression. It is clear that in the coming years improved technologies for targeting oligonucleotides will provide us with an opportunity to modulate aberrantly expressed eRNAs *in vivo*, which could bring forth a new era for enhancer therapy.

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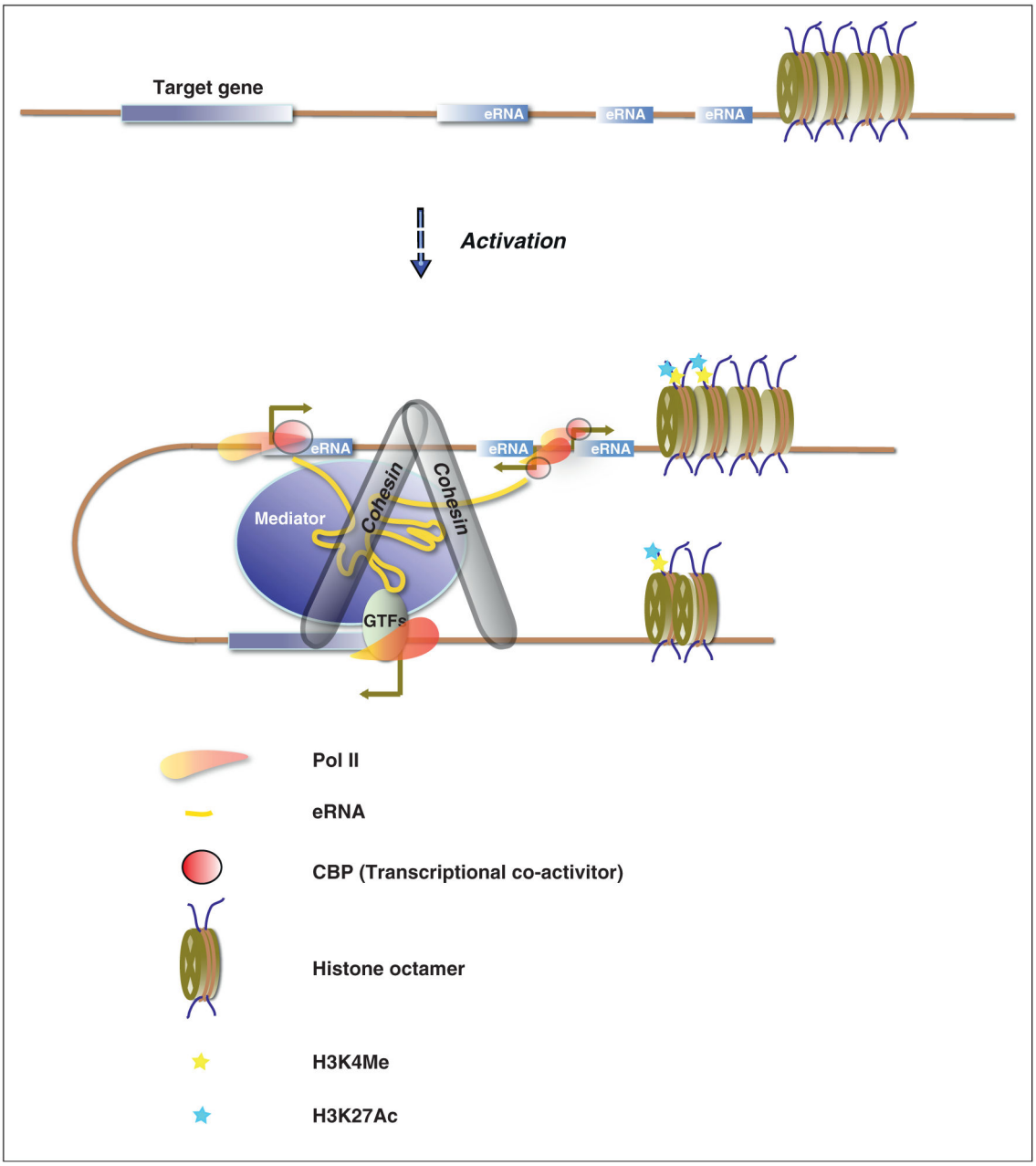

Figure 1.

eRNAs induce chromatin looping and transcriptional activation. Stimulus-induced expression of eRNAs cooperate with transcriptional cofactors such as Mediator and Cohesin to promote chromatin looping and transcriptional activation.