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Review Article

Enhancer RNA: biogenesis, function, and regulation

Rong Ye[1,2], Changchang Cao[1] and Yuanchao Xue[1,2,3]

[1]Key Laboratory of RNA Biology, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China; [3]School of Life Sciences, Henan Normal University, Xinxiang, China

Correspondence: Yuanchao Xue (ycxue@ibp.ac.cn)

Enhancers are noncoding DNA elements that are present upstream or downstream of a gene to control its spatial and temporal expression. Specific histone modifications, such as monomethylation on histone H3 lysine 4 (H3K4me1) and H3K27ac, have been widely used to assign enhancer regions in mammalian genomes. In recent years, emerging evidence suggests that active enhancers are bidirectionally transcribed to produce enhancer RNAs (eRNAs). This finding not only adds a new reliable feature to define enhancers but also raises a fundamental question of how eRNAs function to activate transcription. Although some believe that eRNAs are merely transcriptional byproducts, many studies have demonstrated that eRNAs execute crucial tasks in regulating chromatin conformation and transcription activation. In this review, we summarize the current understanding of eRNAs from their biogenesis, functions, and regulation to their pathological significance. Additionally, we discuss the challenges and possible mechanisms of eRNAs in regulated transcription.

Introduction

Functionally distinct cell types usually exhibit different gene expression patterns. Promoters and enhancers are two principal regulatory elements in genomes that control the spatiotemporal expression of eukaryotic genes [1,2]. Promoters are generally referred to as DNA sequences that determine the transcription initiation site of a gene [2]. In contrast, enhancers are distal DNA elements that activate or increase transcription of linked genes in specific cell types [1]. The first enhancer element was identified when characterizing a 72-bp DNA repeat from the simian virus SV40 [3,4]. This repeat could dramatically boost heterogeneous β-globin gene transcription over 200-fold in transfected HeLa cells and importantly independent of genomic distance and orientation to the promoter [3]. In 1983, the first mammalian cellular enhancer in the intronic region of the immunoglobulin (*Ig*) heavy chain gene was identified [5–7]. This *Ig* enhancer is required to efficiently express *Ig* genes and shows strict tissue or cell-type specificity [5,6]. In addition to typical enhancers, a group of clustered enhancers with broad occupancy by Mediator and master transcription factors (TFs), termed super-enhancers (SEs), were defined in 2013 [8].

Those initial observations and subsequent genome-wide studies have significantly advanced our understanding of enhancers, which generally have the following conspicuous features: (1) the ability to positively boost transcriptions of the linked genes from their correct initiation sites determined by promoters; (2) enhancer activity is independent of orientation and genomic distance to the target gene promoter [3,4]; (3) enhancers usually exhibit high levels of mono- or di-methylation on histone H3 lysine 4 (H3K4me1/me2) but are low or devoid of H3K4me3 [9,10]; (4) in addition to H3K4me1/me2, active enhancer regions are also enriched in H3K27ac marks [11], while inactive or poised enhancers show H3K27me3 enrichment [12]; (5) enhancers are hypersensitive to DNase I digestion, which reflects a decompacted chromatin state [10,13]; (6) enhancers contain specific DNA sequences allowing for the binding of TFs and exhibit enriched co-activators, such as p300 and CBP [10,14,15]. Based on these features, approximately 400 000 candidate enhancers have been identified in the mammalian genome [16].

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Similar to promoters, enhancer regions were also occupied by RNA polymerase II (Pol II) [17,18]. Inspired by the observed transcripts on the locus control regions (LCRs) of the β-globin gene [19–21], researchers suspected that direct Pol II binding at enhancers might also produce noncoding transcripts. The breakthrough occurred in 2010. Two independent studies observed divergent and widespread transcription of active enhancers in neurons and macrophages, and those enhancer-derived RNAs were termed as enhancer RNAs (eRNAs) [22,23]. Unlike eRNAs, a group of long noncoding RNAs (lncRNAs) containing poly (A) tail as well as cap structures but possessing enhancer-like functions were also identified at the same time [24]. Although enhancer DNA itself is essential for transcriptional regulation, depletion of these eRNAs or eRNA-like noncoding RNAs both led to the down-regulation of their linked protein-coding genes [24–27]. These results suggest that eRNAs might be functional molecules rather than merely transcriptional noise resulting from random Pol II initiation at open chromatin regions. In this review, we focused on eRNA and briefly summarized our current understanding of eRNA biogenesis, functions, regulation, and pathological relevance.

Cellular biogenesis of eRNAs

In mammalian cells, enhancers are typically devoid of the nucleosome and thus make their DNA sequences open and accessible for transcriptional machinery assembly [9]. Since the discovery of enhancer elements, many general and specific TFs have been identified to occupy enhancer regions via specific DNA motifs and thereby recruit Pol II (Figure 1) [23,28–31]. Several initial studies in canonical enhancers and recent genome-wide surveys have demonstrated that Pol II at enhancer regions also initiates and elongates to produce eRNAs [19–23]. These enhancer transcripts provide an alternative way to predict active enhancers. A logistic regression model demonstrated that eRNA is more reliable than H3K27ac in defining active enhancers [32], and the bidirectionally capped eRNAs showed a strong correlation with enhancer activities [33]. However, not all enhancers in a cell can generate eRNAs; for example, closed or poised enhancers decorated by H3K4me1 and H3K27me3 marks typically do not produce eRNAs [11]. Therefore, combining eRNA and histone modification can better predict active enhancers. Nuclear run-on followed by 5′ cap sequencing (Gro-cap) and cap analysis of gene expression followed by sequencing (CAGE-seq) suggested that the production of eRNAs is bidirectional with an independent transcription initiation complex assembled in each direction [33,34], and the distance between the two transcription start sites is 110 bp on an average [34]. Such a tight space may exclude two Pol II complexes' simultaneous assembly to initiate transcription in both directions in the same region [34]. However, how these two independent transcription initiation complexes are assembled at active enhancers still needs to be explored.

The C-terminal domain of the Pol II largest subunit contains a heptad repeated (Tyr^1–Ser^2–Pro^3–Thr^4–Ser^5–Pro^6–Ser^7) 52-times in mammals, and the different phosphorylation states of Ser^5 and Ser^2 have been demonstrated to be tightly coupled with transcription initiation and elongation, respectively [44,45]. The Ser^5 phosphorylated Pol II (Ser5P) and another phosphorylated form of Pol II at Tyr^1 are both enriched at promoters and enhancers [28,35], indicating the early transcription at enhancers is analogous to the promoter in mRNA and lncRNA (Figure 1). In contrast, Ser^2 phosphorylation, an indicator of elongation at the protein-coding gene bodies, is lower in the enhancer region [28], indicating that Pol II elongation might be different at enhancer and gene bodies. This difference may be due to the noncontinuous Pol II transcription at enhancers, which contain a high density of poly(A) cleavage sites (PASs) that will lead to early terminations (Figure 1) [34]. Therefore, the length of eRNA is typically short (0.5–2 kb) [23,33]. Like mRNA and lncRNA, eRNA also contains cap structure, demonstrated by the strong Gro-cap and CAGE-seq signals [33,34]. However, distinct from mRNA and lncRNA, eRNA is rarely spliced and mostly nonpolyadenylated [23,33]. Moreover, eRNAs are primarily retained in the nuclear and chromatin fractions, which is different from most mRNAs and lncRNAs [38]. Furthermore, eRNA abundance is 19–34-fold lower than transcripts from neighboring coding genes [33]. Although some abundant eRNAs can reach approximately 70–95 copies per cell [26], many are hard to detect due to persistent degradation by RNA exosomes [40].

TFs have been demonstrated to play critical roles in eRNA biogenesis. The estrogen receptor-α (ERα) is a central TF that regulates cell growth and proliferation by modulating enhancer activity [46]. In this sophisticated transcription program, ERα binds to estrogen-responsive enhancers and organizes a large complex named the mega TF bound in trans (MegaTrans) in MCF-7 breast cancer cells. The MegaTrans complex contains diverse TFs and is required for the recruitment of co-activators, such as p300 and Med1, to activate eRNA transcription [46]. Of note, whether MegaTrans is present in other cell types still needs to be explored. Similarly, during androgen receptor (AR)-dependent eRNA synthesis, transcription-generated torsional stress needs to be relieved by the DNA nicking activity of DNA topoisomerase I (TOP1), which is reported to be a prerequisite for robust eRNA transcription upon stimulation [47].

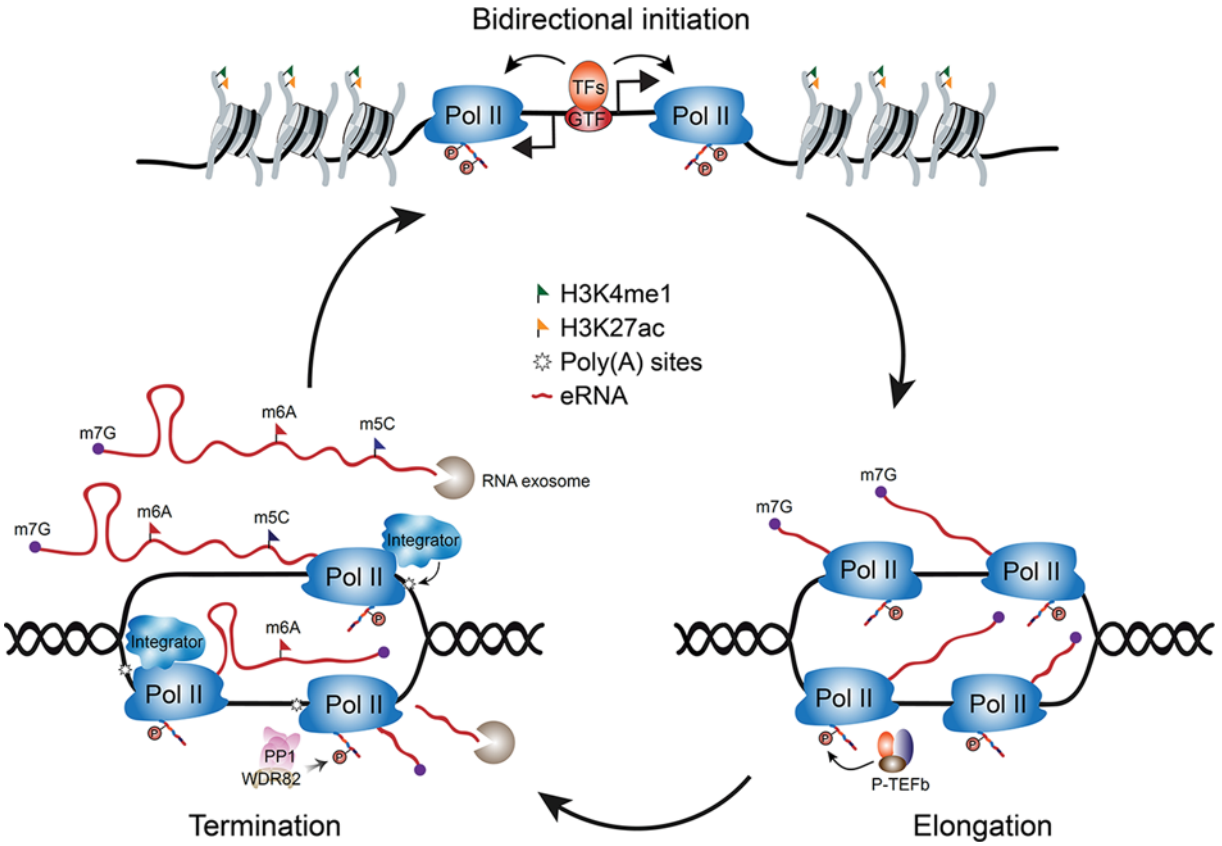

Figure 1. The biogenesis of eRNAs

A schematic diagram of the eRNA transcription process, modifications, and decays. Enhancer regions are enriched with histone H3K4me1 and H3K27ac (green and yellow flags, respectively) marks. After the initial binding of general TFs (GTFs) and other TFs, Pol II will be recruited to the enhancer locus to initiate bidirectional transcription [34]. The CTD of Pol II at the enhancer region is mainly phosphorylated (brown circles) at Tyr^1 and Ser^5 [35,36]. Similar to transcriptions at protein-coding genes, Pol II also undergoes regulated pausing and release at enhancers. The Spt5 and P-TEFb also seem to regulate transcriptional pausing and elongation of eRNAs (red lines) [37]. Transcriptional termination of enhancers is mediated by the Integrator, a large complex containing 12 subunits, probably with the assistance of poly (A) cleavage sites in the nascent eRNA [38]. WDR82 and protein phosphatase 1 (PP1) are also involved in the early termination of eRNAs [39]. After release from chromatin, nuclear RNA exosomes degrade eRNA in a 3′–5′ fashion [40]. RNA modifications such as m5C and m6A are widely present in eRNAs and influence their stability [41–43]. Abbreviation: WDR82, WD Repeat Domain 82.

Like master TF AR and ER, MyoD also occupies thousands of extragenic enhancers and could promote eRNA synthesis [48]. In another example, two studies revealed unexpected roles of p53 in regulating chromatin accessibility and eRNA transcription in response to DNA damage [49,50]. Surprisingly, most p53-activated enhancers do not possess canonical p53-binding sites, and subsets of them are activated by a p53-induced lncRNA named LncRNA activator of Enhancer Domains (LEDs) [50]. Unlike the aforementioned positive regulators, nuclear receptors, Rev-Erbs, repress eRNA transcription by recruiting NCoR/HDAC3 corepressor complexes to Rev-Erb response enhancers [25]. In macrophages, overexpressing Rev-Erbs resulted in decreased eRNA production, while Rev-Erbs knockout significantly increased eRNA transcription [25]. In addition to protein factors, 7SK small nuclear RNA (snRNA) could also occupy enhancers genome-wide and restrict eRNA synthesis by changing chromatin structures through interacting with the BAF chromatin-remodeling complex [51].

Like U1 and U2 snRNA genes, the transcriptional termination of eRNA is also regulated by a multisubunit complex called Integrator, which interacts with the Pol II CTD and cleaves the 3′ end of primary eRNA transcripts to facilitate its release from transcribing Pol II [38]. Integrator depletion reduces the stimulus-induced steady state of eRNAs and abrogates long-range enhancer–promoter chromatin looping [38]. However, whether and how Integrator regulates the coupling between 3′-end termination and eRNA stability is still unknown. In another example, the adaptor protein

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WD Repeat Domain 82 (WDR82) can recognize and target the nuclear protein phosphatase 1 complex to the initiating Ser^5 Pol II at enhancer regions, leading to the dephosphorylation of Pol II and early transcriptional termination of eRNA synthesis [39]. Together, these results collectively indicate that TFs, co-activators, and corepressors can regulate eRNA biogenesis at multiple stages of the transcription cycle.

RNA modifications in eRNAs are also tightly linked to their metabolism and function. For instance, peroxisome proliferator-activated receptor-γ co-activator 1 α (PGC-1α) could interact with methyltransferase 7 (NSUN7) to deposit 5-methylcytosine (m5C) chemical modification on eRNAs to enhance their stability. The depletion of NSUN7 caused reduced m5C and eRNA levels, therefore diminishing eRNA-targeted gene expression [41]. Another transcriptome-wide mapping of m6A in human tissues revealed 1908 enhancer-derived lncRNAs containing m6A modifications, suggesting that m6As may also be associated with the metabolism of eRNAs [42]. Recent work further demonstrated that m6A modifications on chromatin-associated regulatory RNAs such as eRNAs and repeat RNAs can facilitate the decay for a subset of them, and therefore decrease chromatin accessibility and repress transcription in mouse embryonic cells [43]. However, whether it is a general phenomenon and how m6A, as well as other RNA modifications, regulate eRNA biogenesis still await further work.

Functional roles of eRNAs

eRNAs were initially thought to be transcriptional byproducts because of their low abundance, lack of RNA processing, and short half-life. However, many pieces of evidence have firmly contradicted this traditional view. Splitting the HS2 enhancer or insertion of transcriptional terminator could both abolish the synthesis of enhancer transcripts and the transcription of the linked β-globin gene [52,53]. These results indicate that enhancer element itself and enhancer-initiated eRNAs might both contribute to the gene activation. However, emerging evidence supports the direct involvement of eRNA in transcription activation. Knocking down eRNAs with small interfering RNAs, short hairpin RNAs, or locked nucleic acid oligonucleotides in different studies reduced the transcription of eRNA-linked promoter genes [25–27], demonstrating the functional importance of the eRNA transcript itself. Importantly, large-scale genome-wide association studies (GWASs) have revealed that approximately 90% of single nucleotide polymorphisms are localized at intronic or intergenic regions [54], where enhancers abundantly exist. In the human brain, a robust set of enhancer regions that express eRNA are enriched for genetic variants associated with autism spectrum disorders (ASDs) [55]. Additionally, global fine-mapping in 21 autoimmune diseases found that approximately 60% of the causal variants are mapped to immune-cell enhancers [56], and these enhancers often produce eRNAs upon immune stimulation (Figure 2). Furthermore, mutations located in eRNA-transcribing enhancers impair ACTRT1 expression and cause basal cell carcinoma (BCC) [57]. These findings demonstrate the potential causal relationship between eRNAs and diverse diseases.

The transcripts of eRNAs themselves play important roles in genomic stability (Figure 2). The transcription process unwinds the double-stranded DNA template and generates lagging RNA transcripts. Sometimes, the RNA strand is tightly base-paired with template DNA to form a three-stranded structure called R-loop, which needs to be resolved in a timely manner [65,66]. Accumulated and prolonged R-loops usually lead to genomic instability [67]. It has been described in mouse embryonic stem cells and B cells that RNA exosome-mediated eRNA degradation might maintain genomic integrity by impeding R-loop formation [40]. This regulation could occur via post-transcriptional degradation or early termination of eRNA synthesis. In addition, divergently transcribed genomic regions, such as SEs, are preferred substrates of activation-induced cytidine deaminase (AID) [59,60], a critical DNA mutator initiating somatic hypermutation (SHM) and class switch recombination (CSR) for antibody maturation in B cells (Figure 2). AID mistargeting at oncogenes generally leads to chromosomal translocations and tumorigenesis. Importantly, AID targeting to enhancer regions is dependent on eRNA and RNA-binding protein ROD1 [61]. Furthermore, clustered enhancers at specific genomic regions usually trigger convergent transcription, in which RNA polymerase random collision might also cause genomic instability [59,68].

The crucial roles of eRNA in diverse diseases make it ideal as a potential therapeutic target. Many AR-regulated eRNAs are up-regulated in castration-resistant prostate cancer (CRPC) cells and patient tissues [69]. One of them, PSA eRNA, containing a TAR RNA-like motif that resembles the secondary structure of the 3′ end of 7SK snRNA, can directly bind to Cyclin T1 of the positive transcription elongation factor b (P-TEFb) complex. This binding will further compete P-TEFb away from the inhibitory 7SK/HEXIM1/2 complex and promote target gene transcription in *cis* or *trans* by increasing the Ser^2 phosphorylation of Pol II [69]. As PSA eRNA is necessary for CRPC cell growth, it may be a valuable therapeutic target in the clinic. Additionally, eRNAs expressed from enhancers in the striatum are reduced in Huntington's disease (HD) mice compared with control mice, resulting in the down-regulation of striatal neuron identity genes [70]. In a different genetic mouse model of human cardiac hypertrophy, inhibiting hypoxia-inducible

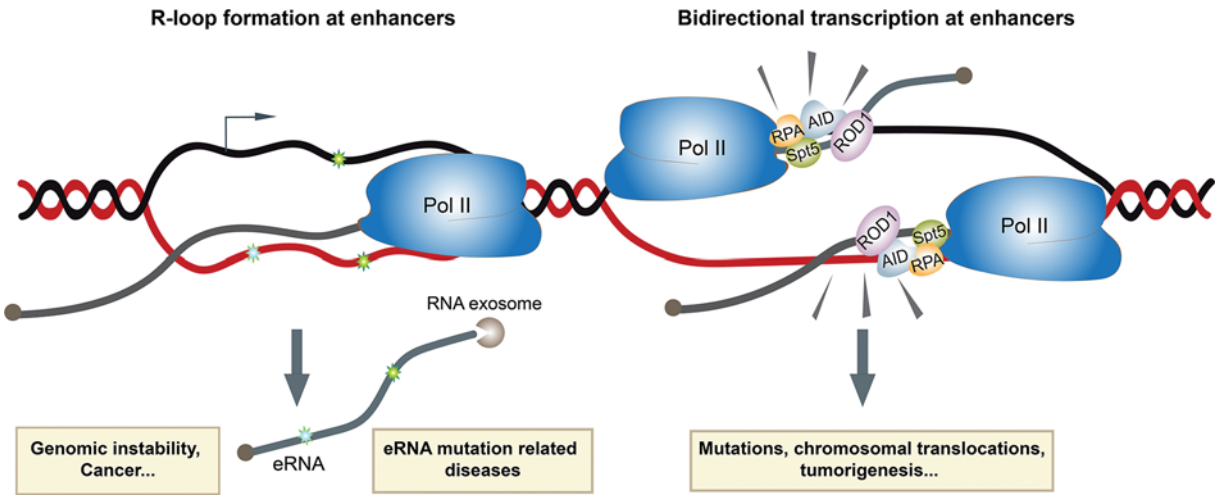

Figure 2. The biological function of eRNAs

Genetic variants (stars) residing in enhancers can pass the mutation information to eRNA and cause diverse human diseases. At many enhancer loci, actively transcribed eRNAs preferentially form stable R-loops with template DNA. Prolonged and unresolved R-loops usually cause DNA damage, chromosome translocations, and genome instability [58]. In B cells, many intragenic SEs undergo convergent transcription to produce large amounts of eRNAs [59,60]. These eRNAs can be bound by the RNA-binding protein ROD1 via CU-rich motifs, and such binding will further recruit the DNA mutator AID to the locus, leading to oncogenic translocations and tumorigenesis [61]. Additionally, several factors, such as Spt5, RPA, and 14-3-3, also play critical roles in AID targeting [62–64]. Abbreviation: AID, activation-induced cytidine deaminase.

eRNA (HERNA)1 with antisense oligonucleotides prevented stress-induced pathological hypertrophy and dramatically increased overall survival [71]. In another study, an eRNA, AP001056.1, was significantly associated with survival in patients with squamous cell carcinoma of the head and neck (SCCHN) [72]. AP001056.1-higher patients showed a better survival rate than AP001056.1-lower patients [72]. These findings suggest that eRNAs are functionally important and could serve as therapeutic targets of diverse diseases.

Regulatory mechanism of eRNAs

Multiple-related mechanisms have been proposed for eRNA function, including eRNAs regulating long-range enhancer-promoter looping, chromatin accessibility, and transcriptional elongation. In MCF-7 breast cancer cells, ERα occupies approximately 7000 enhancer regions and increases eRNA production globally in an estrogen-dependent manner [26]. Several induced eRNA transcripts seem to be necessary to strengthen enhancer–promoter looping, which could be further stabilized by the interactions between ERα and cohesion [26]. Similarly, in prostate cancer cells, AR also bound enhancer regions and promoted eRNA transcription in an androgen-dependent manner [73]. One of the eRNAs, KLK3e, located upstream of Kallikrein-related peptidase 3, could facilitate the proximal interactions between the KLK3 enhancer and the KLK2 promoter and thus promote the transcriptional activation of KLK2 [73]. Such an eRNA-dependent chromosomal looping model appears to be generally applicable [26,38,74], but the detailed working mechanism is still unclear. As RNA inside the cells usually associates with different RNA-binding proteins (RBPs) [75], therefore, eRNA-interacting RBPs might also contribute to the proximal looping between the enhancer and promoter. Indeed, Integrator, Mediator, heterogeneous nuclear ribonucleoprotein (hnRNP) U, and hnRNPK showed critical roles in eRNA-mediated long-range chromosomal looping [38,74,76,77]. Correspondingly, depletion of RAD21 (a subunit of cohesion), MED1/12 (subunits of Mediator), Integrator, hnRNPU, and hnRNPK all abolished enhancer–promoter looping and gene activation [26,38,74,76,77].

In addition to modulating enhancer–promoter looping, eRNAs also regulate gene expression by adjusting chromatin accessibility, chromatin compartmentalization, and histone modifications. During muscle cell differentiation, two enhancers at the *MYOD* locus were transcribed individually to generate ^{CE}eRNA and ^{DRR}eRNA [48,78]. ^{CE}eRNA is necessary for maintaining the chromatin accessibility of the nearby *MYOD* locus. In contrast, ^{DRR}eRNA interacts with the cohesin complex and occupies the *myogenin* locus to activate its transcription by increasing chromatin accessibility. In developing T cells, the transcription of ThymoD eRNA repositions the Bcl11b enhancer from the lamina

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to the nuclear interior, juxtaposing the enhancer and promoter into a single-loop domain to activate Bcl11b transcription [79]. Histone acetylation and CBP/p300 co-activator binding is a well-known signature of active enhancers. An intriguing study demonstrated that eRNAs could bind CBP to stimulate its histone acetyltransferase activity [80]. The increased CBP/p300 activity causes more H3K27ac and H3K18ac at the enhancer and even the target promoter. The knockdown of different eRNAs led to decreased H3K27ac and repressed transcription of target genes. This result suggests that eRNA synthesis and co-activator binding are mutually synergetic processes.

eRNAs can also regulate gene expression by recruiting the transcription machinery. During skeletal muscle cell differentiation, SE-generated seRNA-1 directly interacts with hnRNPL to facilitate Pol II binding and H3K36me3 deposition at the nearby *myoglobin* locus [81]. Apart from Pol II binding, an earlier study illustrated that eRNAs facilitate Pol II release by acting as a decoy for the negative elongation factor (NELF) complex [27]. In response to neuronal activity, eRNAs from the enhancer of Arc promoted the dissociation of NELF from paused Pol II to induce the productive elongation of neuronal immediate early genes. Knockdown of eRNAs impaired NELF release and inhibited gene activation but unexpectedly did not affect enhancer–promoter looping [27]. Another elegant mechanism was proposed as TF trapping in studying gene activation by Yin-Yang 1 (YY1) [82], which could bind promoter-proximal and promoter-distal elements. In this process, the bidirectionally transcribed promoter RNA and eRNA not only bound YY1 but also enhanced YY1 occupancy at these regulatory elements to promote gene transcription. This eRNA-mediated trapping mechanism seems to be general, as it works by artificially tethering *Arid1a* RNA to six distinct enhancer regions.

Enhancers are typically occupied by transcription co-activators BRD4 and MED1, which can bridge interactions between TFs and Pol II to promote transcription initiation [83]. Unexpectedly, both BRD4 and MED1 have intrinsically disordered regions (IDRs) and can form phase-separated droplets in the cell [84]. Such liquid–liquid phase separation can organize individual TFs, co-activators, and Pol II into membrane-less biomolecular condensates at SE regions to drive cell identity gene transcription. Whether eRNA participates in this nucleation process remains unknown. We recently found that SE RNAs exhibited strong proximal interactions with promoter RNA, eRNAs, and nascent precursor mRNA (pre-mRNA) transcripts (Figure 3) [76]. Because such intricate RNA–RNA interactions are mediated by RBPs, which typically contain IDRs, those RBP-mediated intermolecular RNA–RNA interactions may also play a role in determining enhancers and promoter communications. This RNA-mediated phase separation model might explain the formation, function, and properties of SEs (Figure 3). In addition, it has been demonstrated that m6A sites in mRNAs could promote phase separation [85], and whether eRNAs containing m6A modification have similar roles still needs to be investigated. Similarly, GATA3 and ERα, two key TFs that are recruited to the MegaTrans enhancers, can also phase separate *in vitro* and *in vivo* [86]. Importantly, the presence of eRNAs can promote phase separation of the two TFs *in vitro*. These initial studies collectively indicate that eRNA may play crucial roles in transcriptional activation by regulating phase separation at noncoding genomic regions.

RNA–RNA interactions in transcription activation

Like microRNAs, snRNAs, and lncRNAs, eRNA might also achieve its regulatory functions by directly base-paired or indirectly interacting with other RNA molecules. Both of these two scenarios are very likely for a subset of promoter upstream antisense RNAs (uaRNAs or PROMPTs) considering their proximity to enhancers during transcriptional activation. To directly mapping of RNA–RNA interactions in cells, several state-of-the-art methods have been developed for identifying RNA duplexes, including CLASH, hiCLIP, PARIS, and RPL [89–92]. Although these methods have significantly advanced our understanding of RNA–RNA interactions inside the cells, none of them observed interactions between eRNA and uaRNAs. This limitation is probably because the expression levels of eRNA and uaRNAs are too low to be detected. By combining proximity ligation and chimeric RNA enrichment, we recently developed an RNA *in situ* conformation sequencing (RIC-seq) technology to globally map RNA–RNA spatial interactions [76]. Unexpectedly, we detected widespread interactions between eRNA and uaRNA. We further demonstrated that these eRNA–uaRNA interactions could be used to faithfully assign enhancer and promoter connectivity. To our surprise, approximately 90% of the promoter-linked genes showed reduced transcription upon the corresponding eRNA knockdown. Moreover, some of these eRNA–uaRNA interactions appear to be critical for maintaining long-range enhancer–promoter looping. The observed interactions between eRNA and uaRNA not only provide a robust way to assign enhancer–promoter pairing but also open the door for further dissecting the detailed mechanisms of eRNA in transcriptional activation.

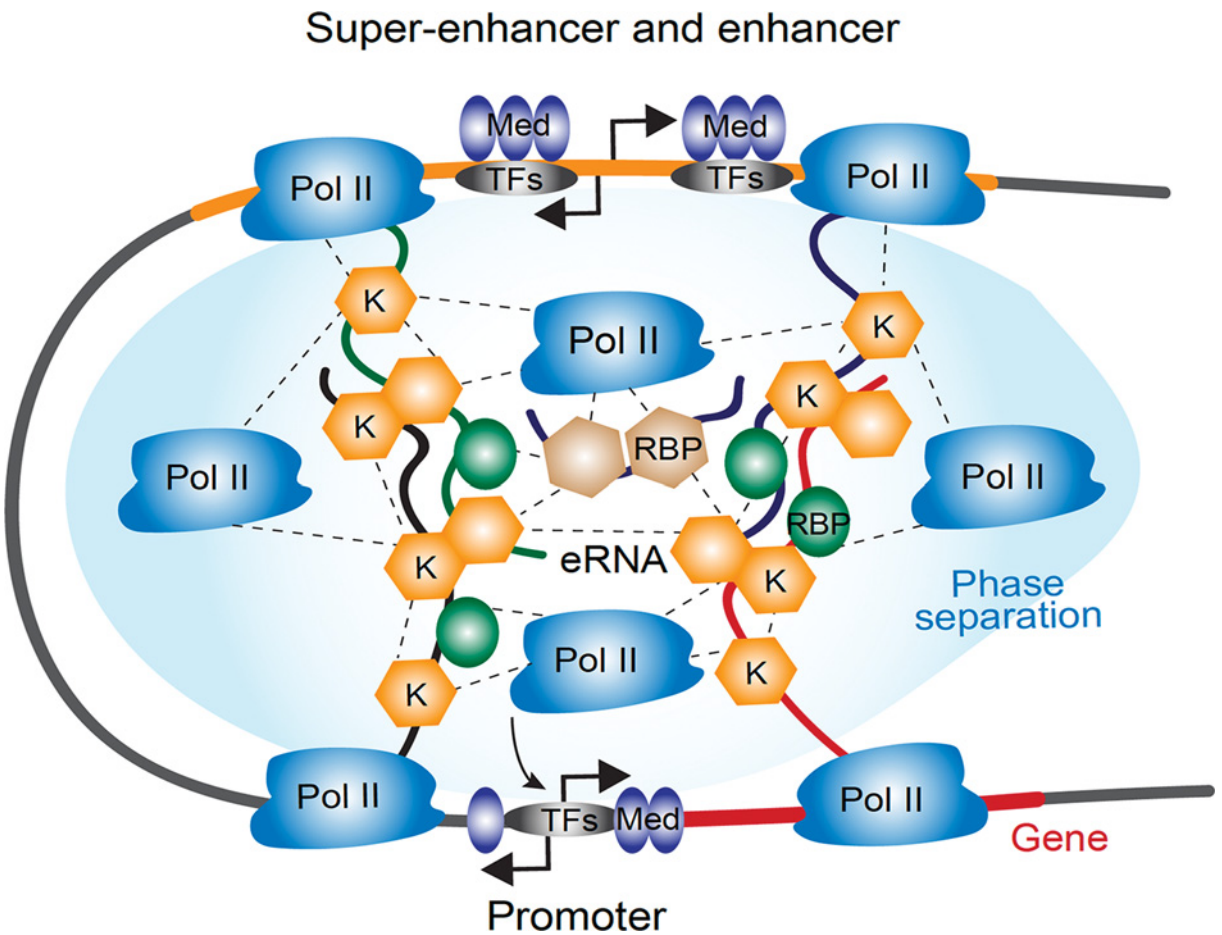

Figure 3. An eRNA-mediated phase separation model for transcriptional activation

Gene activation is dependent on the long-range DNA looping between the transcription apparatus assembled at enhancers and TFs at the core promoter. Transcription co-activator Mediator (Med) can interact with cohesion to modulate DNA looping [87]. Enhancers and promoters are divergently transcribed, generating eRNAs, promoter RNAs, and sense pre-mRNAs [34]. eRNAs extensively interact with promoter-derived noncoding RNAs and pre-mRNAs [76], and such interactions are mostly dependent on RBPs such as hnRNPK. Therefore, knockdown of eRNA or hnRNPK can significantly disrupt DNA looping frequency. Recently, both mediators and Pol II have been shown to form phase-separated condensates at a SE locus [88]. This phase separation model perfectly explained the transcriptional busting ability of enhancers. Importantly, it explained why a single enhancer or SEs could simultaneously activate multiple genes. We recently found that eRNA can interact with promoter RNAs to regulate long-range enhancer–promoter looping and through the oligomerization of hnRNPK to deliver Pol II from SEs to the promoter locus [76]. Considering that RNA could boost the phase separation of the transcription apparatus at enhancer regions [86], it is tempting to propose an eRNA-mediated phase-separated model to explain the interactions between eRNA, promoter RNA, and RBPs in transcription activation. Of note, this figure is modified from our recently published paper [76].

Conclusions and perspectives

It is estimated that over 6 million enhancers may be present in the human genome based on experimental analysis of histone modifications, chromatin accessibility, cap structures, and chromatin conformations [93]. The identification of eRNA from active enhancers is a radical breakthrough in the field, forcing us to think deeper about the regulatory precision and complexity of the transcription program. Although many studies have demonstrated the crucial roles of eRNAs in regulating long-range enhancer–promoter looping, several fundamental questions remain: (1) How does eRNA influence chromatin looping? (2) Are there specific features such as coexist sequence motif present between paired enhancer–promoters at the RNA level? (3) How does eRNA activate transcription of the paired promoter genes? (4) How do pairwise interacting RNAs between promoters and enhancers contribute to disease? (5) How

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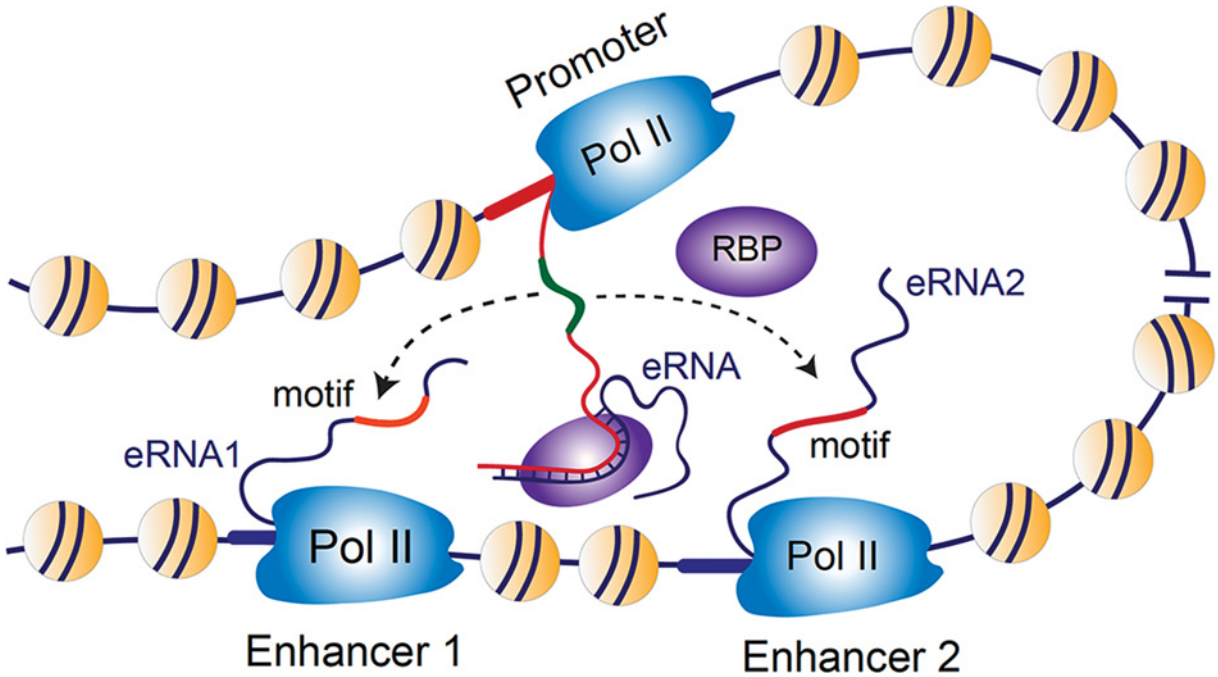

Figure 4. RNA–RNA interactions in transcriptional regulation

Some unknown features might be present at eRNAs and uaRNAs to regulate enhancer and promoter communication. RBPs may also play crucial roles in regulating such communications by selectively interacting with specific motifs in paired eRNA–uaRNAs.

generally can RBPs regulate enhancer and promoter communication via shared motifs in eRNA and uaRNA (Figure 4)?

Given that eRNAs have low abundance and are unstable, developing more sensitive methods to unbiasedly identify eRNA targets would help answer the questions mentioned above. As enhancers are physically close to promoters during gene activation and pre-mRNA is more abundant than eRNAs, global mapping of pre-mRNA to enhancer DNA contacts is also an efficient way to deduce eRNA targets [94]. Additionally, developing *in vivo* imaging methods to monitor the interaction dynamics between eRNA, uaRNA, and genomic loci would help understand the sequential events during transcriptional activation. For example, does looping drive RNA–RNA interactions or do RNA–RNA interactions drive loop formation. Furthermore, some enhancers, especially SEs, can proximally interact with many promoters. Therefore, developing unbiased methods to map individual eRNA- or SE RNA-regulated RNA networks may help us to determine the communication principles between enhancers and promoters.

Summary

- Bidirectionally transcribed eRNAs are the hallmark of active enhancers.
- Most eRNAs are not transcriptional byproducts but play crucial roles in transcriptional activation.
- An eRNA-mediated phase-separated model may contribute to transcription activation via the interplay among eRNA, promoter RNA, and RBPs.
- The identification of eRNA enables us to appreciate the complexity and precision of the transcription program in development and diverse diseases.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

R.Y., C.C., and Y.X. all participated in preparing this manuscript.

Abbreviations

AID, activation-induced cytidine deaminase; AR, androgen receptor; BAF, Barrier-to-autointegration factor; CAGE-seq, cap analysis of gene expression followed by sequencing; CRPC, castration-resistant prostate cancer; CBP, CREB-binding protein; CTD, carboxy-terminal domain; eRNA, enhancer RNA; Gro-seq , Global Run-On sequencing; H3K4me1/me2, mono- or di-methylation on histone H3 lysine 4; H3K27ac, acetylation of histone H3 lysine 27; IDR, intrinsically disordered region; *Ig*, immunoglobulin; LCR, locus control region; lncRNA, long noncoding RNA; MegaTrans, mega TF bound in trans; m5C, 5-methylcytosine; NCoR/HDAC, Nuclear Receptor Corepressor/ Histone deacetylase; NELF, negative elongation factor; PAS, poly(A) cleavage site; Pol II, RNA polymerase II; pre-mRNA, precursor mRNA; P-TEFb, positive transcription elongation factor b; RBP, RNA-binding protein; SE, super-enhancer; snRNA, small nuclear RNA; TF, transcription factor; YY1, Yin-Yang 1.

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