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Chromatin-enriched lncRNAs: a novel class of enhancer RNAs

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Abstract

Long noncoding (lnc)RNAs are postulated to control diverse biological processes by modulating transcription, yet for most lncRNAs evidence supporting this function has been lacking. A new report describes the role of a novel class of lncRNAs—chromatin-associated enhancer RNAs or cheRNAs—in the regulation of proximal gene expression.

An increasing array of non-protein-coding RNAs characterizes eukaryotic genomes, including that of humans^{1–4}. Over the course of last 15 years, first through microarray screens and subsequently through RNA sequencing (RNA-seq), a large number of lncRNAs have been annotated in a diverse set of species^{5–7}. lncRNAs are postulated to control all aspects of DNA metabolism, most notably transcription, and to influence cellular differentiation, organismal development, and disease progression^{1,8–11}. However, most lncRNAs have not been tested for function in gene regulatory processes. More recently, a subclass of lncRNAs has been found to be transcribed from many enhancers, termed enhancer RNAs or eRNAs^{12,13}. eRNAs require a greater depth of sequencing to be detected reliably, as they are either transcribed at low levels or rapidly degraded¹⁴. Recently, Ruthenburg and colleagues discovered a novel class of putative eRNAs that remain bound to chromatin and are not easily solubilized, which they termed chromatin-enriched enhancer RNAs or cheRNAs¹⁵.

cheRNAs show several molecular characteristics that are distinct from those of eRNAs. Whereas most eRNAs are bidirectionally transcribed from the prototypical enhancers, cheRNAs show a specific strand bias^{15,16}. Moreover, eRNAs are marked by the histone H3K4 monomethylation (H3K4me1) and H3 lysine 27 acetylation (H3K27ac)¹², whereas cheRNAs are associated with H3K4me3 (ref. 15). Finally, cheRNAs are longer than eRNAs (median length of ~2,000 as compared to ~350 nucleotides)^{13,15}. In this issue of *Nature Structural & Molecular Biology*, Werner, Ruthenburg and colleagues functionally characterize several cheRNAs¹⁷.

The Ruthenburg group had previously discovered enrichment of cheRNAs in the non-soluble as compared to the soluble chromatin fraction of RNAs by means of rigorous sub-nuclear fractionation coupled with RNA-seq of HEK293 cells¹⁵. A majority of these cheRNAs remain attached to chromatin through interactions with RNA polymerase II (Fig. 1).

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Intriguingly, cheRNA expression was positively correlated with the expression of neighboring protein-coding genes. In the current study, through a series of biochemical and genetic experimentations, the authors demonstrate that cheRNAs are expressed in a cell-type-specific manner, and that these RNAs promote changes in chromatin architecture and thereby contribute to the expression of nearby genes. cheRNA profiling in three divergent cell lines, HEK293, K562, and H1hESCs, showed that proximity to cheRNAs was a better predictor of *cis*-gene expression than features such as chromatin modification signatures or the expression of eRNAs or other lncRNAs.

To functionally test cheRNAs, Werner *et al.* used CRISPRi to inhibit the transcription of three different cheRNAs that are highly expressed in K562 cells¹⁸. Truncated transcription of the cheRNAs abrogated the induction of the nearest coding genes, providing evidence that cheRNAs are required for robust expression of neighboring genes. The *cis*-limited function of cheRNAs suggested a model whereby the cheRNAs localize to a neighboring gene and thus control its expression. In agreement with this, RNA fluorescence *in situ* hybridization (RNA-FISH) probing of the cheRNA PVT1 to its target gene *MYC* showed colocalization of PVT1 with the *MYC* transcript. This result indicated that the PVT1 cheRNA bridges its genomic site to the target gene *MYC*. CRISPRi of another cheRNA, HIDALGO, demonstrated that it is required for full stimulation of the fetal hemoglobin subunit gamma-1 (*HBG1*) gene during the differentiation of K562 cells toward erythroid lineages.

To distinguish whether the act of transcription or the HIDALGO cheRNA itself activates *HBG1* expression, Werner and colleagues degraded the HIDALGO RNA using anti-sense oligonucleotides and found a significant decrease in *HBG1* transcription, suggesting that cheRNAs themselves play a functional *cis*-regulatory role. To investigate a potential mechanism of HIDALGO RNA function, the authors performed chromatin conformation capture (3C), and they discovered that a *HIDALGO* transcription start site contacts the *HBG1* promoter, a loop that is diminished upon CRISPRi depletion of HIDALGO. Relatedly, cheRNA loci are enriched at topologically associating domain boundaries. This observation is consistent with a recent model suggesting that noncoding RNA transcription can serve as central points for chromosome domain contacts¹⁹. The authors also analyzed the evolution of cheRNAs and found that they are enriched for type I mammalian transposons, implying that these transposable sequences may be the origin of the transcription-enhancing cheRNAs.

In summary, Werner *et al.* convincingly show that the cheRNA class of lncRNAs can function to activate gene expression of proximal genes. Nevertheless, not all cheRNAs may function similarly. For example, one cheRNA examined by the authors, PAINE, did not significantly influence the expression of the closest neighboring gene. It remains possible, however, that the PAINE cheRNA may alter expression of one or more genes that lie at a distance. Another possibility is that a subset of cheRNAs may fine-tune or perhaps even inhibit nearby gene expression. Future investigations will most certainly further refine the mechanisms by which this novel class of lncRNAs regulates transcription, genomic configuration, and cell fate.

References

1. Deveson IW, Hardwick SA, Mercer TR & Mattick JS Trends Genet. 33, 464–478 (2017). [PubMed: 28535931]
2. Liu G, Mattick JS & Taft RJ Cell Cycle 12, 2061–2072 (2013). [PubMed: 23759593]
3. Cabili MN et al. Genes Dev. 25, 1915–1927 (2011). [PubMed: 21890647]
4. Derrien T et al. Genome Res. 22, 1775–1789 (2012). [PubMed: 22955988]
5. Kapranov P et al. Science 296, 916–919 (2002). [PubMed: 11988577]
6. Kapranov P et al. Genome Res. 15, 987–997 (2005). [PubMed: 15998911]
7. Djebali S et al. Nature 489, 101–108 (2012). [PubMed: 22955620]
8. Lai F et al. Nature 494, 497–501 (2013). [PubMed: 23417068]
9. Gayen S, Maclary E, Buttigieg E, Hinten M & Kalantry S Cell Rep. 11, 1251–1265 (2015). [PubMed: 25981039]
10. Gayen S, Maclary E, Hinten M & Kalantry S Proc. Natl. Acad. Sci. USA 113, E309–E318 (2016). [PubMed: 26739568]
11. Schmitt AM & Chang HY Cold Spring Harb. Perspect. Med 10.1101/cshperspect.a026492 (2017).
12. Kim TK et al. Nature 465, 182–187 (2010). [PubMed: 20393465]
13. Andersson R et al. Nature 507, 455–461 (2014). [PubMed: 24670763]
14. Li W, Notani D & Rosenfeld MG Nat. Rev. Genet 17, 207–223 (2016). [PubMed: 26948815]
15. Werner MS & Ruthenburg AJ Cell Rep. 12, 1089–1098 (2015). [PubMed: 26257179]
16. Melgar MF, Collins FS & Sethupathy P Genome Biol. 12, R113 (2011). [PubMed: 22082242]
17. Werner MS et al. Nat. Struct. Mol. Biol 24, 596–603 (2017). [PubMed: 28628087]
18. Qi LS et al. Cell 152, 1173–1183 (2013). [PubMed: 23452860]
19. Melé M & Rinn JL Mol. Cell 62, 657–664 (2016). [PubMed: 27259198]

Figure 1.

A schematic of cheRNA function. cheRNAs are a new class of lncRNAs that are tightly associated with chromatin. A majority of cheRNAs (red) are tethered to their site of synthesis via RNA polymerase II and regulate expression of nearby genes. Right, an example of a cheRNA that bridges regulatory sequences through chromosome loops to induce transcription of the neighboring protein-coding gene (green). Histone octamers are shown as purple cylinders.