Enhancer-derived RNAs: 'spicing up' transcription programs

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Recent reports established transcription of enhancer-derived RNAs (eRNAs), while the evidence for their functional significance remained mostly speculative. Two recent reports published in *Nature* (Lam *et al*, 2013; Li *et al*, 2013) offer the first functional evidence for eRNA transcripts. These analyses further reveal an unexpected level of specificity in the regulation of adjacent mRNAs by eRNAs.

Enhancers are genomic elements that have been implicated in the regulation of gene transcription for many years. They function by promoting transcriptional activity of promoter regions, generally from significant distances away, and in many cases they are the primary transcription factor binding site. Understanding the complex relationship between promoters and their enhancers has been a major focal point in recent times. The number of enhancers in a cellular context has been estimated to be 55 000 (Heintzman *et al*, 2009) and there is tissue-specific enhancer activity (Visel *et al*, 2009), implying that there is plasticity in determining what elements become enhancers within a specific cellular context.

A surprising observation arising from analysis of enhancer elements was the finding that eRNAs are transcribed from the

genomic sequence encompassing the enhancer. These findings resulted from the use of novel technical approaches for assessing non-coding RNAs, including GROseq (Core *et al*, 2008). This methodological approach was exploited to identify cell lineage-specific eRNAs. As examples, analysis of membrane depolarization in primary neuronal cultures revealed approximately 2000 eRNAs that were produced at enhancers, with a coincident enrichment of H3K4me1 marks and binding of both the co-activator CBP and RNA PolIII. In many cases, the eRNAs produced from an individual enhancer were bidirectional and these correlated with expression levels of the adjacent mRNA (Kim *et al*, 2010), suggesting that expression of the eRNA and the neighbouring mRNA are linked. In oestrogen-stimulated breast cancer cells, a large number of eRNAs were induced within tens of minutes of ligand exposure from enhancer elements that were bound by oestrogen receptor (ER; Hah *et al*, 2011). These findings suggest that hormone stimulation results in expression of coding mRNAs that use enhancer elements for optimal transcriptional activity, while eRNAs are simultaneously transcribed from the distal enhancer elements themselves. In a similar system, activation of the androgen receptor in prostate cancer cells results in

Figure 1 (A) Activation of transcription by eRNAs in the MCF-7 breast cancer setting is mediated by binding of ER α to enhancer regions, producing sequence-specific eRNAs that mediate looping from the enhancer region to the distal transcription start site by stabilizing Cohesin. (B) In macrophages, eRNAs produced from an enhancer site play a role in transcription of an adjacent gene. Binding of Rev-Erb proteins that bring in HDACs repress the production of these eRNAs and thus lead to the inactivation of the adjacent gene.

expression of numerous eRNAs from AR-bound enhancer elements (Wang *et al.*, 2011). Additionally, the tumour suppressor p53 has been reported to regulate production of eRNAs, which are required for the production of neighbouring mRNA transcripts (Melo *et al.*, 2013). As such, eRNAs appear to be a relatively common phenomenon, yet it was unclear if they served a functional role.

Two recent papers (Lam *et al.*, 2013; Li *et al.*, 2013) expand our understanding of the functional role of eRNAs. Both reports provide data to show that eRNA transcripts have working roles in the subsequent regulation of adjacent coding mRNA genes. An unexpected finding is that there is exquisite specificity between an individual eRNA and regulation of the gene proximal to the enhancer that produced the eRNA in the first place. The two reports show that both positive and negative regulation of eRNAs can occur, with consequences on the regulation of proximal coding genes. Li *et al.* (2013) examined the production of eRNAs from enhancers of ER α -regulated genes in hormone-treated MCF-7 breast cancer cells, while Lam *et al.* (2013) focused on the role of specific eRNAs at Rev-Erb- α - and Rev-Erb- β -bound enhancers during gene repression in macrophages. Both groups verified that the actual strand-specific sequence of the eRNA is important for gene regulation rather than the enhancer element simply functioning as a classic transcriptional enhancer for the adjacent coding mRNA.

Li *et al.* (2013) focused on a number of specific eRNAs produced from enhancers of oestrogen-regulated genes. They showed that inhibition of eRNAs, using multiple approaches, did not result in global changes in gene expression programs, but instead resulted in discrete changes in the mRNA adjacent to the enhancer sequence that produced the eRNA. They conclude that *trans* effects of individual eRNAs are limited and that eRNAs elicit their effect within the localized genomic environment from where they are transcribed. Since it is known that chromatin loops form between enhancer elements and the promoters of the target genes, Li *et al.* (2013) hypothesized that individual eRNA transcripts may be involved in the formation or maintenance of chromatin–chromatin interactions. Inhibition of eRNAs produced from two classic ER target gene enhancers (involved in regulation of the *NR1P1* and *GREB1* genes) resulted in the loss of the chromatin loops between the enhancers and the target genes at both genomic regions. In addition, individual eRNAs were

shown to interact with subunits of the cohesin complex, which has a well-established role in the formation of chromatin interactions (Kagey *et al.*, 2010). The authors suggest that eRNAs are required for association of cohesin subunits, since eRNA inhibition depleted binding of both Rad21 and SMC3, two cohesin subunits. Interestingly, Lam *et al.* (2013) reported that 76% of enhancer elements that are bound by Rev-Erb in macrophages can produce eRNAs, with half of these generating bidirectional eRNA transcripts. Following Rev-Erb binding, eRNA transcription is blocked and this correlates with inhibition of expression of the adjacent mRNA. This mechanism is at least partially specific, since the same observations were not seen when investigating another transcription factor, PU.1, which is involved in macrophage differentiation. This study also identified a specific histone signature at enhancers that are involved in the production of eRNAs, namely high H3K4me1 and low H3K4me3.

These findings clearly show that eRNAs are functionally important, they act in *cis* on adjacent target genes and they contribute to enhancer activity in a novel and unexpected way (Figure 1). The exact mechanism of gene regulation at nearby promoters by these short RNA sequences has yet to be elucidated. One possibility is that as eRNAs are transcribed, they become incorporated in the transient chromatin interactions that occur between enhancers and promoters, subsequently assisting in stabilization of these associations for optimal transcriptional activity of the coding gene. This would imply that there is an opportunistic interaction mediated via the geographical proximity between the enhancer/promoter interface and the eRNA that is transcribed from the enhancer element. An alternative hypothesis is that sequence specificity defines associations between an eRNA, the enhancer that bore the eRNA and the gene that is in *cis*. As such, a remaining question is how an eRNA has such unexpected specificity in the target gene it regulates. Nevertheless, these two recent reports provide the first evidence that specific eRNA transcripts produced from enhancer elements are functionally important in the regulation of cell-specific gene expression events.

Conflict of interest

The authors declare that they have no conflict of interest.

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