RESEARCH HIGHLIGHT

eRNAs reach the heart of transcription

Cell Research (2013) 23:1151–1152. doi:10.1038/cr.2013.97; published online 23 July 2013

Recently, various studies shed light on the functional significance of enhancer RNAs. Two recent studies published in *Nature* by Li *et al.* and Lam *et al.* highlight the importance of these newly characterized RNA molecules and their key role in controlling transcriptional programs.

Enhancers are short genomic sequences that regulate transcription of remotely located target genes. Specific epigenetic signatures, such as histone methylation and acetylation, normally define enhancers. Primarily, active enhancers are marked by high level of histone 3 lysine 4 monomethylation (H3K4me1), low level of H3K4 trimethylation (H3K4me3) [1, 2] and high level of histone 3 lysine 27 acetylation (H3K27Ac) [3]. Additionally, a landmark paper by Kim *et al.* [4] described enhancer RNAs (eRNAs), a new class of non-coding RNAs (ncRNAs), that are produced from polymerase II-bound enhancers. This study has unraveled a complex connection between eRNA-producing enhancers and their target genes. Most significantly, the level of expression of eRNAs positively correlated with the expression of nearby coding genes [4]. Follow-up studies revealed the potential and functional importance of eRNA-producing enhancers by studying key transcription factors [5, 6]. Our lab, for example, has demonstrated the binding of the tumor suppressor p53 to specific enhancer regions (termed p53-bound enhancer regions or p53BERs). By using chromosome conformation capture (4C) combined with deep sequencing, we observed that p53BERs interacted intrachromosomally with neighboring

genes, thereby conveying a long-distance p53-dependent transcriptional activation [6]. However, to date, the global functional relevance of transcription at enhancers remains largely unclear. Two recent studies tackle this subject and show that eRNAs can either positively or negatively impact the transcriptional regulation of nearby coding genes [7, 8].

Li and colleagues set out to investigate the DNA binding profile of oestrogen receptor α (ER- α) in human breast cancer cells [7]. By using Global Run-On sequencing (GRO-Seq) on 17 β -oestradiol (E2)-treated cells, they observed a correlation between ER- α -bound enhancers, eRNA induction, and oestrogen-upregulated expression of neighboring genes. To investigate whether eRNA production from ER- α -bound enhancer impacts chromatin structure, they applied a three-dimensional DNA selection and ligation (3D-DSL) procedure. Interestingly, they found that E2 treatment intensified the frequency of interactions between enhancers and promoters. Supporting this notion, targeting eRNAs with antisense oligos reduced the number of enhancer-promoter interactions. As several reports have already indicated cohesin as a key component for the formation and stabilization of chromatin structures [9], the authors performed immunoprecipitation with antibodies against Rad21 (one subunit of the cohesin complex) followed by deep sequencing (ChIP-seq). Indeed, a subset of Rad21-binding sites was clearly overlapping with E2-induced eRNA-producing enhancer domains. This suggested an interaction between eRNAs and the cohesin complex, which was then confirmed by

RNA immunoprecipitation experiments. Altogether, Li *et al.* propose that many enhancers produce eRNAs that attract cohesin complexes to stabilize the chromatin structure between enhancers and promoters.

With a similar approach, Lam *et al.* [8] endeavored to elucidate the molecular mechanisms by which the nuclear receptors Rev-Erb- α and Rev-Erb- β regulate macrophage gene expression. Using ChIP-Seq the authors established genome-wide binding profiles of these two factors. Detailed analysis revealed that the majority of the shared bound regions of Rev-Erb- α and Rev-Erb- β are located more than 1 kb away from any transcription start site (TSS) and mainly at enhancer domains (as defined by specific epigenetic marks). Lam and colleagues carried on with their investigation by performing GRO-Seq in Rev-Erb- α and Rev-Erb- β double knockout macrophages, which revealed that most of Rev-Erb-bound enhancer regions were producing eRNAs. To explore whether Rev-Erbs directly control eRNA production at bound enhancer regions, as a mean to regulate neighboring gene expression, the authors intersected Rev-Erb loss- and gain-of-function GRO-Seq data with Rev-Erb- β binding profile. This analysis revealed an inverse correlation between Rev-Erb- β binding and the levels of eRNAs. Thus, differently from the activating function of ER- α , Rev-Erbs seemed to silence eRNA production in bound enhancers. This effect was to a large extent mediated by recruiting the NCoR-HDAC3 repressive complexes [10]. Altogether, Lam and colleagues pinpointed the ability of Rev-Erb to

repress macrophage gene expression by downregulating eRNA production at distant and specific enhancer sites. The next important question was whether the production of sequence-specific eRNAs or the transcription at enhancer regions is the cause of transcriptional regulation of target genes. In other words, do eRNAs possess transcriptional enhancement activity? Melo *et al.* [6] recently demonstrated that eRNAs produced from p53BERs support the activation of gene expression [6]. Both Li *et al.* [7] and Lam *et al.* [8] extended these observations to eRNAs regulated by ER- α and Rev-Erbs. Using various reporter constructs, they demonstrate that the production of sequence-specific eRNAs appears to be a prerequisite for the emergence of enhancing activity (Figure 1). Thus, both studies not only confirm the role of eRNAs in transcription enhancement, but also pinpoint sequence requirement and suggest impact

on chromatin conformation.

However, numerous intriguing questions remain unanswered. First, the properties or rules governing the specificity of action of the eRNAs are still undefined. One enticing idea involves the potential role of eRNA structure in mediating protein recruitment to specific promoter targets to activate transcription. This type of activation was proposed for long intergenic non-coding RNAs (lincRNAs) [11] and might also occur with eRNAs. Alternatively, sequence homology between eRNAs and promoters could dictate the formation of a RNA-DNA hybrid complex at specific targets, leading to the subsequent alteration in chromatin structure and transcription activity at target genes. Additionally, recent studies have demonstrated the role of the mediator protein complex in the transcription activation by non-coding RNAs (e.g., ncRNA-activating) [12],

raising the possibility that eRNAs might also interact with the mediator complex to control transcription activity. This would suggest that despite the different epigenetic features of eRNAs and ncRNA-activating, the mediator may provide a common mechanistic foundation for both types of transcription regulators. Altogether, recent new reports on eRNA production and function reveal novel insights into the understanding of how enhancers work. As eRNAs are targetable, they may present a point of vulnerability, which can be beneficial for enhancer silencing and gene expression regulation *in vivo*.

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Figure 1 A model showing the influence of eRNAs on the expression of neighboring genes. Key transcription factors can activate (ER- α and p53) or repress (Rev-Erb proteins) enhancer domains by inducing or silencing eRNA production. While activated eRNAs can lead to a greater stabilization of the DNA looping (eRNA-cohesin interaction), transcriptional repressors (Rev-Erb- α/β) can achieve their function through epigenetic silencing (HDAC deacetylation) of enhancer domains and the repression of eRNA production. The two scenarios have opposite impact on the transcriptional regulation of neighboring genes. α or β , Rev-Erb- α or - β .