

'Lnc'-ing enhancers to MYC regulation

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Long noncoding RNAs (lncRNAs) are emerging as important functional components in the establishment of long-range chromosomal interactions. In a recent paper published in *Cell Research*, Xiang *et al.* provide mechanistic insight into this phenomenon by characterizing the role of *CCATI-L*, a colorectal cancer-specific lncRNA, in intra-chromosome looping between the *MYC* gene promoter and distal upstream enhancer elements that regulate *MYC* transcription.

Enhancer elements are the principal means through which cell type/context-specific gene expression is achieved. Enhancers contain binding sites for multiple transcription factors that work in concert to regulate the activity of specific gene promoters. Enhancers can be separated from the genes that they regulate by large genomic distances, but are brought into close physical proximity through a process known as chromosome looping. The mechanisms through which chromosome looping between enhancers and promoters is regulated are incompletely understood. However, several reports have described widespread transcription occurring within enhancer elements and have suggested that the resulting noncoding RNA transcripts may be involved in enhancer-promoter interactions [1-5].

In a recent paper published in *Cell Research*, Xiang *et al.* [6] characterize one such enhancer-derived long noncoding RNA (lncRNA), *CCATI-L*, and conclude that it does indeed have a functional role in chromosome looping at the *MYC* locus. The authors first identified *CCATI-L*, an alternative isoform of the

previously described lncRNA *CCATI*, after performing RNA-Sequencing (RNA-Seq) in colorectal cancer (CRC) patient tissue samples. As compared to normal tissue samples, *CCATI-L* expression levels were significantly elevated in CRC tissue. Furthermore, *CCATI-L* expression was detected in several CRC-derived cell lines but not in non-CRC cell lines, underscoring the specificity of *CCATI-L* in CRC.

The *CCATI-L* transcript is encoded within an enhancer located 515 kb upstream of the *MYC* gene. In general, enhancer-derived RNAs are not spliced or polyadenylated; however, *CCATI-L* contains two exons and a poly-A tail [1, 3, 7]. The authors used a combination of cell fractionation and FISH experiments to show that *CCATI-L* is enriched in the nucleus and localized at its site of transcription in CRC-derived cells. Subsequent knockdown experiments using antisense oligonucleotides (ASOs) revealed that depletion of *CCATI-L* resulted in the reduction of *MYC* transcription. However, transient overexpression of *CCATI-L* had no effect on *MYC* expression. Collectively, these observations suggest that *CCATI-L* is involved in *MYC* regulation and that it functions *in cis* at the *MYC* enhancer.

Dissecting the mechanistic aspects of *cis*-functioning lncRNAs can be difficult because experiments require the perturbation of endogenous loci. Xiang *et al.* overcame this hurdle using genome editing to engineer two unique CRC-derived cell lines with modifications at the endogenous *CCATI-L* locus. In one line a CMV promoter and an *egfp* gene were inserted upstream of *CCATI-L*, resulting in the overex-

pression of an *egfp-CCATI-L* fusion transcript. In the second line the *egfp* gene was followed by a double poly-A site, which allowed *egfp* expression as a control but prevented overexpression of the downstream *CCATI-L* transcript. The authors found that overexpression of *egfp-CCATI-L* resulted in increased *MYC* expression, supporting the role of *CCATI-L* in *MYC* regulation and further suggesting that *CCATI-L* functions *in cis* at that *MYC* enhancer.

To investigate the interactions between the *CCATI-L*-encoding enhancer and the *MYC* promoter, the authors next utilized chromosome conformation capture (3C). The 3C assay is a proximity ligation-based approach that can detect interactions between genomic regions that are distant from one another in sequence space but in close physical proximity in the nucleus. Using this approach the authors validated interactions between the *CCATI-L* locus and the *MYC* promoter. The authors also detected interactions between an additional *MYC* enhancer and both the *MYC* promoter and the *CCATI-L* locus. Subsequent DNA FISH experiments confirmed that the *MYC* gene, the *CCATI-L* locus, and the additional enhancer co-localize in the nucleus of CRC-derived cells. Moreover, RNA FISH revealed that the *egfp-CCATI-L* RNA was also localized to these sites.

The authors turned to publically available ChIP-Seq datasets to gain insight into potential mechanisms through which *CCATI-L* might be involved in chromosome looping at the *MYC* locus and noticed that CTCF-binding sites overlap the *MYC* promoter as well as both upstream enhancers. The authors

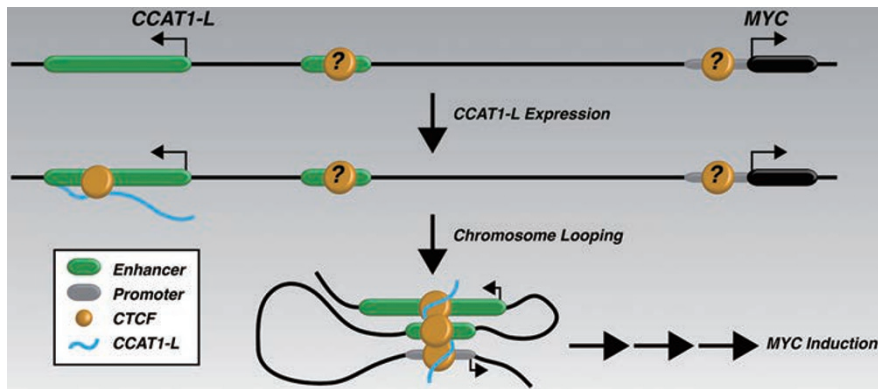


Figure 1 *CCATI-L* is transcribed from a distal enhancer of *MYC*, associates with CTCF, and mediates chromosome looping between the *MYC* promoter and its upstream enhancers.

depleted CRC-derived cells of either *CCATI-L* or CTCF and observed a reduction in chromosome looping between the *MYC* promoter and its enhancers, which was accompanied by a decrease in *MYC* expression. Furthermore, a combination of biotin-labeled RNA pull-down and RNA immunoprecipitation experiments were used to characterize a direct interaction between *CCATI-L* and CTCF. The authors concluded the study by demonstrating that depletion of *CCATI-L* from CRC-derived cells resulted in a reduction in CTCF occupancy across the *MYC* locus.

In summary, Xiang *et al.* provide compelling evidence to support a model whereby an enhancer-derived lncRNA, *CCATI-L*, associates with CTCF and the resulting interaction facilitates chromosome looping across the *MYC* locus (Figure 1). The findings of Xiang *et al.* touch on a widely debated question in the study of enhancer-derived RNAs: are the RNAs transcribed from

enhancers functional, are they simply a byproduct of open chromatin, or is it the act of their transcription that is required for enhancers to become accessible? In the case of *CCATI-L* the answer remains unclear, although a strong argument could be made for the act of transcription being the key mechanistic feature. For example, increased transcription through the *CCATI-L* locus led to an increase in enhancer activity. However, the interactions between the *MYC* promoter and its upstream enhancers, as well as the occupancy of CTCF across these regions, remained largely intact following the depletion of *CCATI-L* RNA. Regardless of how *CCATI-L* functions, Xiang *et al.* have illuminated previously unappreciated aspects of *MYC* regulation by distal enhancer elements, an advancement that has important implications in cancer biology.

The observations of Xiang *et al.* also complement several recent reports on lncRNAs that facilitate chromosomal

interactions and contribute to the proper establishment of nuclear architecture [8, 9, 10]. These reports include additional lncRNAs that are involved in intra-chromosomal looping as well as lncRNAs that mediate interactions between multiple chromosomes [2, 4, 5, 9, 10, 11]. Collectively, these examples indicate that lncRNAs may be critical components of the molecular mechanisms important for shaping the three-dimensional structure of the genome.

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