Making Sense of Antisense Transcription

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A striking finding from genome-wide analysis of transcription is that a large fraction of many eukaryotic genomes is transcribed into long noncoding RNAs (lncRNAs). While in a few cases specific functions have been ascribed to lncRNAs, whether the majority of lncRNAs are functional has been the subject of ongoing debate (Ulitsky and Bartel, 2013). Aside from the question of function, how the expression of lncRNAs is regulated is unclear. To dissect mechanisms that regulate the expression of lncRNAs, graduate student Eric Alcid in the laboratory of Dr. Toshio Tsukiyama (Basic Sciences Division) focused on antisense lncRNAs (ASlncRNAs), which overlap mRNA coding regions but are transcribed in the opposite direction. Using a novel genetic screen in budding yeast, he identified many new repressors of ASlncRNA transcription, including components of four highly conserved chromatin remodeling complexes. This extends the authors' previous work showing that the chromatin remodeling factor Isw2 represses the transcription of ASlncRNAs from the 3' ends of protein-coding genes (Whitehouse *et al*., 2007) and shows that chromatin remodeling is a major player in the regulation of ASlncRNA transcription.

The authors first created a genetic system to screen for regulators of ASlncRNA transcription. They hypothesized that, since ASlncRNAs often overlap mRNA coding regions, that RNAi, which uses double stranded RNA as a template to target mRNAs for degradation, would process mRNA:ASlncRNA hybrids and stabilize ASlncRNAs. Thus, they reconstituted RNAi in budding yeast. While reconstitution of RNAi alone did not lead to growth defects, genetic crosses to mutants known to stabilize ASlncRNAs resulted in variable degrees of growth impairment, indicative of widespread mRNA degradation. Having confirmed that their system was sensitive to increases in ASlncRNA levels, they used a technique called synthetic genetic array (SGA) analysis, allowing them to cross their RNAi-reconstituted strain to a collection of ~5,000 non-essential gene deletion mutants and screen for growth defects. The identified numerous genes involved in biological processes related to RNA metabolism as well as regulations of mRNA transcription. Additionally, a number of chromatin regulators were among the hits, including subunits of four chromatin remodeling complexes: Isw2, Swr1, Ino80, and Rsc. The genetic interactions between RNAi and chromatin remodelers were subsequently confirmed using a different genetic background and additional mutants.

Having identified chromatin remodelers as putative lncRNA repressors, the authors next sequenced RNA from yeast strains carrying mutations in each remodeling complex. This revealed a total of 1,799 ASlncRNAs that were upregulated in the absence of chromatin remodelers. Each remodeling complex targeted mostly unique sets of ASlncRNAs, perhaps reflecting their varying effects on chromatin structure, and suggesting that there are multiple ways in which chromatin structure can repress ASlncRNA transcription.

As sequencing of RNA from chromatin remodeler mutants cannot distinguish between ASlncRNAs directly and indirectly repressed by remodelers, the authors used genome-wide chromatin immunoprecipitation (ChIP) data to identify ASlncRNAs whose transcription start sites are bound by remodelers. This analysis showed that 814 (~45%) of all remodeler-repressed ASlncRNAs are bound by remodelers at their start sites. The authors designated ASlncRNAs directly repressed by remodelers CRRATs (chromatin remodeling-repressed antisense transcripts).

Lastly, the authors addressed the question of ASlncRNA functionality. To do this, they identified CRRATs whose upregulation was associated with decreases in their overlapping mRNAs. This analysis identified 259/814 (~31.8%) CRRATs associated with mRNA decreases, suggesting that ASlncRNA repression of mRNA transcription is a widespread mechanism for transcriptional repression.

"Our study has two potential, big-picture implications: 1) The cell devotes numerous resources to attenuating noncoding RNA levels genome-wide and 2) mRNA control through regulation of the overlapping noncoding RNA is far more common that previously appreciated," said Alcid. Furthermore, given that the chromatin remodelers identified in this study are evolutionarily conserved, chromatin-level repression of ASlncRNA expression may also be a conserved regulatory mechanism.

Alcid E, Tsukiyama T. 2014. ATP-dependent chromatin remodeling shapes the long noncoding RNA landscape. *Genes Dev*28(21):2348-2360.

See also: Ulitsky I, Bartel DP. 2013. lincRNAs: Genomics, Evolution, and Mechanisms. *Cell* 154(1):26-46.

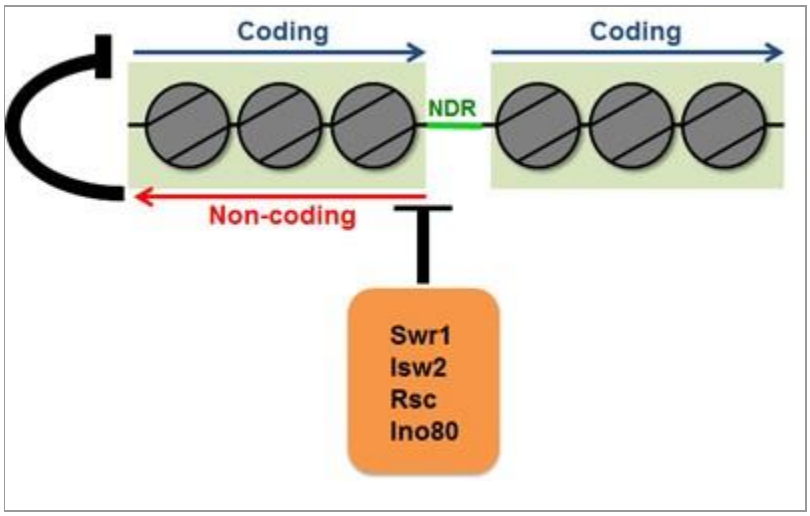

Image provided by Eric Alcid

The Swr1, Isw2, Rsc, and Ino80 chromatin remodeling complexes repress the expression of antisense long noncoding RNAs (ASlncRNAs) that overlap mRNA coding regions and repress mRNA expression.