Sense and antisensibility

May 16, 2016

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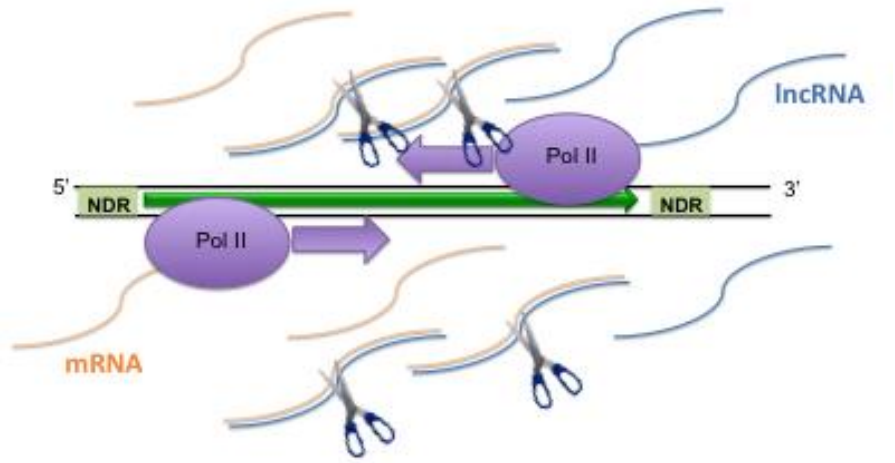

Antisense long, non-coding RNAs (ASlncRNAs) associate with mRNAs to form double stranded RNAs (dsRNAs) that are degraded by the exosome as well as the RNAi machinery. Cleaving dsRNAs is a key way cells protect themselves from pathogen invaders. However, researchers from the Tsukiyama Lab have found that yeast harbor and can tolerate high levels of ASlncRNAs and that these high levels of ASlncRNAs are not due a loss of function of the exosome or RNAi alone. Instead, these species have evolved increased expression of lncRNAs due to mutations in additionally, as yet unknown RNA regulatory processes.

Image provided by Dr. Tsukiyama.

Genes are transcribed into messenger RNAs (mRNAs), which are the templates for molecular machines in the cell, called ribosomes, to coordinate production of the encoded protein. The transcription of DNA into RNA can occur in two orientations, called sense and antisense. When genes are transcribed into RNAs that code for a protein, the direction of transcription is called "sense" and the transcript is called mRNA. When transcription occurs in the opposite direction and cannot be translated into protein, the transcription is called "antisense" and the RNA is "non-coding". Surprisingly, antisense transcription appears widespread in many organisms, including humans, where antisense transcripts have been identified for more than 30% of annotated sense transcripts[2]. The origin and function of long, non-coding RNAs (lncRNAs) has remained mysterious, although a handful of lncRNAs have been shown to regulate gene expression in response to environmental cues. Almost nothing is known about the evolution of lncRNA expression, the study of which may uncover important clues to reveal its function in cells.

In an investigation recently published in *Nature Structural & Molecular Biology*, former graduate student Dr. Eric Alcid in Dr. Toshi Tsukiyama's Laboratory (Basic Sciences Division) quantified lncRNA levels within different species of yeast and discovered that lncRNA expression is dramatically increased in yeast that do not have a functional RNA interference (RNAi) pathway, a processing machinery that breaks down double-stranded RNAs (dsRNAs). After first finding that levels of well-studied lncRNAs have increased expression in yeast species lacking RNAi, Dr. Alcid conducted genome-wide strand-specific RNA sequencing to identify and quantify lncRNA expression in different yeast species, either possessing or lacking a functional RNAi pathway based on research from other groups. The dramatic difference in lncRNA expression between species suggests that RNAi restricts lncRNA levels, likely because RNAi can destabilize dsRNAs created when lncRNAs and mRNAs overlap.

In addition to the overall number of antisense transcripts, Dr. Alcid analyzed the length of RNA transcripts in different species of yeast. He found that yeast lacking RNAi had longer lncRNAs on average, overlapping more with mRNAs. In order to directly test whether RNAi restricts the expression of lncRNAs, Dr. Alcid genetically engineered a yeast species lacking RNAi, *S. cerevisiae*, to reconstitute RNAi. When RNAi was restored in this yeast, he found that the number of lncRNA transcripts decreased. However, the level of lncRNAs was still higher than the level of lncRNAs in yeast species that normally retain RNAi such as *N. castellii*. Additionally, Dr. Alcid found that inactivating RNAi in yeast which normally retain RNAi such as *N. castellii* did not cause them to express as many lncRNAs as (RNAi negative) *S. cerevisiae*. Therefore, the presence or absence of RNAi alone cannot explain the differences in lncRNA levels among yeast species.

A conserved enzyme complex called the exosome degrades the majority of lncRNAs and mutating the exosome leads to the stabilization of RNAs called cryptic unstable transcripts (CUTs). The scientists wondered whether, similar to the increase in "stable" lncRNA transcripts in yeast since the loss of RNAi, expression of short unstable transcripts (CUTs) also increased in these yeast species. They analyzed genome-wide lncRNA levels in *S. cerevisiae* strains with and without an exosome mutation. They found that lncRNA expression increased when the exosome was mutated. Interestingly, although mutating both the RNAi and the exosome machineries in the *N. castellii* species also lead to an increase in lncRNA expression, this level of lncRNAs still did match the high levels seen in *S. cerevisiae* with an exosome mutation. These results suggest that, regardless of their stability, lncRNAs are far more robustly expressed in yeast species that have lost RNAi. This is the first time RNAi has been shown to correlate with the evolution of lncRNAs, a very recently discovered class of RNA transcripts. These findings also highlight the need for additional

research into what currently unidentified molecular pathways, in addition to RNAi and exosomal processing, must restrict antisense transcription in order for mRNAs to remain stable.

This research was funded by the National Institutes of Health.

1. Alcid EA, Tsukiyama T. 2016. "Expansion of antisense lncRNA transcriptomes in budding yeast species since the loss of RNAi." *Nature Structural & Molecular Biology*. 23(5):450-455. doi:10.1038/nsmb.3192.

2. Steinmetz LM, Pelechano V. 2013. "Gene regulation by antisense transcription." *Nature Reviews Genetics*. 14:880-893. doi:10.1038/nrg3594.