Study identifies new mechanism of RNA degradation in plants

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The totality of RNA molecules in an organism at any one time is the product of a delicate dance. Genes must be "turned on," or expressed, in order to turn DNA into RNA and then that RNA into proteins that accomplish an organism's physiological needs. But, just as important, those RNA transcripts must be cleared away once they are no longer required.

University of Pennsylvania researchers have new insights into the latter process, identifying a novel mechanism by which RNA molecules are degraded. The study, led by Brian D. Gregory, an associate professor in Penn's Department of Biology, and postdoctoral fellow Xiang Yu in the same department, provides the first evidence that RNA degradation can occur in the same space and at the same time that RNA translation is occurring in plants. Building on work that identified a similar process in yeast, the Penn research suggests that this may be an evolutionarily conserved process.

"RNA degradation is, in my opinion, an overlooked aspect of gene regulation," Gregory said. "The point being that it's just as important as gene expression, which we all think about. Our contribution here is to show that degradation doesn't have to happen separately from translation. We see that some percentage of RNA is being degraded as the ribosome moves down the length of the transcript. It's happening co-translationally."

The work appears in the journal *Plant Cell*.

Gregory and Yu collaborated on the paper with fellow lab members Matthew R. Willmann, the co-first author, and Stephen J. Anderson.

Prior to this study, scientists were aware of two ways that messenger RNA can be degraded, one starting at the beginning, or 5-prime end of an RNA transcript, and another starting at the end, or the 3-prime side. In both cases the degradation mostly occurs in the cytoplasm.

Recent studies in yeast identified a pathway of RNA degradation that broke from this mold, occurring while the RNA transcript was still attached to a ribosome, the molecular machinery responsible for translating RNA into proteins.

Gregory's lab focuses on RNA regulation in plants, and the Penn scientists wanted to explore whether the process the yeast researchers had observed was also found in plants.

To do so, the team used a technique developed by Gregory as a postdoc, known as genome-wide mapping of uncapped and cleaved transcripts, or GMUCT, which identifies only those RNA molecules that are either cleaved or in the process of degrading. Examining flower bud RNA from Arabidopsis, they performed GMUCT, sorting out the suspected co-translationally degrading transcripts from transcripts that were likely cleaved by another process governed by microRNAs by looking for the same signal the yeast researchers had seen: a cleavage event occurring 16 to 17 nucleotides upstream of messenger RNA stop codons, where translation ceases. This location lines up with the 5-prime edge of where a ribosome would be overlaying the transcript during translation termination.

"We see that the degrading RNA is associated with the ribosome in plants, just as it had in yeast," Yu said. "Many molecular events are coupled together. For example, gene transcription is coupled with

splicing. So this is another example of a coupling, where two molecular events are linked in space and time."

To further explore this coupling event, the researchers interrogated the results of the GMUCT, looking for examples of where the ribosome paused during the degradation/translation process. They noted that the ribosome paused in particular at stop codons, and, to their surprise, they found a distinction between the three different sequences that code for stop codons.

"Until now, everyone has thought that it doesn't matter which stop codon you use, that they all do the same thing," Gregory said. "From our data, it looks like the kinetics of the interaction between ribosome and transcript are distinct for each type of stop codon, or that there may be a distinct complex of proteins associated with each."

The finding, the researchers said, could suggest that translation efficiency might be regulated by which of the three stop codons is present.

Their results also detected signs of a regulatory region in the untranslated region of RNA transcripts, regions known as upstream open reading frames, or uORFs. They detected these active areas by noting a peak of cleavage points at specific places in the 5-prime untranslated regions of transcripts and a pausing at the stop codons present in the uORFs.

"A lot of these active upstream open reading frames regulate the downstream open reading frames in a very tissue-specific and developmentally-specific manner, so we can use our approach to find out which ones are active in whatever tissue you're profiling," Gregory said.

The researchers performed experiments with mutant plants to gain a sense of the molecular players involved in co-translational RNA decay.

Their work found roles for XRN4, an enzyme known to catalyze RNA degradation, and ABH1, part of the nuclear mRNA cap-binding complex, which is involved in many biological processes including messenger RNA stability and splicing and the initial round of protein translation. Their findings suggest that this process may be particularly important in regulating genes involved in response to stress, such as temperature.

"This process looks like a very potent way to get rid of the transcripts that you need to get rid of in order to have a proper stress response," Gregory said.

"It seems reasonable that co-translational decay would be a more efficient, faster process than some of the classical, known degradation pathways," Yu said.

Because the researchers observed that transcripts regulated by microRNA-mediated translation inhibition were more likely to undergo co-translational decay, they are envisioning a model by which these may be responsible for directing their target transcripts into this process of co-translational decay, perhaps even physically shuttling them through this process.

To follow up on their findings, Gregory's group would like to examine whether the processes they observed, co-translational degradation and the distinct interactions they observed based on different stop codons, are tissue-specific processes, that is, whether they appear in other plant tissues beyond flower buds.

And they would also like to probe whether the process extends beyond yeast and Arabidopsis plants, perhaps even to humans.

More information: Xiang Yu et al. Genome-Wide Mapping of

Uncapped and Cleaved Transcripts Reveals a Role for the Nuclear mRNA Cap-Binding Complex in Co-translational RNA Decay in Arabidopsis, *The Plant Cell* (2016). DOI: 10.1105/tpc.16.00456