Researchers have uncovered one way plants respond to hormonal cues

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Plants with a mutation in the gene for an enzyme called DX01 (on right) were stunted in their growth compared to normal plants. The researchers found this owed to a breakdown in the process by which messenger RNA is tagged for degradation. Credit: Courtesy of the Gregory laboratory

Just like other organisms, plants must respond dynamically to a variety of cues over their lifetime. Going through different developmental stages, or altering their form in response to a drought or drastic

temperature change requires altering which of their genes are expressed into proteins and when those processes occur.

In a new paper in *Developmental Cell*, a research team led by Penn biologists Brian Gregory and Xiang Yu identified a mechanism by which plants can conduct this agile regulation of gene expression. They unpacked the details of a process whereby hormone signaling triggers the removal of a structure called nicotinamide adenine dinucleotide (NAD^+) from one end, called the 5' end, of certain messenger RNA (mRNA) molecules, the transcripts that give rise to proteins. When present, these caps direct the cell to break down the associated mRNA transcript, ensuring that its corresponding protein is not made.

"We saw changes in the level of mRNA NAD^+ capping occurring in different plant tissues and in different developmental stages," says Gregory, senior author on the paper and an associate professor in the School of Arts & Sciences' Department of Biology. "This appears to be a potentially quick on/off switch that plants can use to regulate their RNA levels."

"Researchers working on mammalian cells had identified an enzyme that appears to perform an analogous action, removing these NAD^+ caps," says Yu, a postdoctoral researcher in Greogry's lab and the paper's first author. "Ours is the first study to show this process in a whole, living organism."

This work has its origins in preliminary findings that Gregory's lab generated close to a decade ago. While teaching a class on RNA, Gregory had shared with his students a paper about a yeast version of the plant protein DX01, an enzyme now known to be responsible for removing NAD_+ from mRNA.

"I became really intrigued about what it was doing in eurkaryotes," he

says. At that time, his lab grew plants with a DX01 mutation and found that their growth was stunted, their leaves were pale green, their development was delayed, and they had defects in fertility.

"I thought, 'This is cool, we need to work on this,'" Gregory recalls.

Pursuing it, they found that the mutants had an abundance of small RNAs, molecules often associated with silencing the expression of other RNA molecules. But ultimately they couldn't piece together a sensible story of how the mutation was causing small RNAs to accumulate, and the work stalled.

It stalled that is, until a few years ago, when other scientists who work on mammalian RNA regulation began publishing work showing that mammalian cells possess DX01 as well, and that it could recognize and remove NAD^+ caps.

With this new understanding of DX01's role, Gregory, Yu, and colleagues decided to pick their own work back up. By studying plants, the group could take the findings in mammals a step further, looking in vivo, at how the enzyme was acting in a live, growing organism.

The researchers first confirmed that DX01 acted similarly in plants as in mammals, removing the NAD^+ from mRNA transcripts. Plants lacking DX01 developed the problems Gregory had seen years earlier: stunted growth and development. They also used a technique to isolate and sequence only the NAD^+-capped mRNAs and found that mRNA transcripts with NAD^+ caps occurred frequently for those encoding proteins related to stress response, as well as those involved in processing NAD^+ itself. Further analysis confirmed that the NAD^+ cap made mRNAs more likely to be broken down.

To follow up on the clues pointing to an involvement in stress response,

the team applied varying levels of a plant stress hormone, abscisic acid, to plants with or without a functioning DX01. Plants with a mutant DX01 did not appear to be affected by the changing hormone concentration, while those with a functional DX01 were, pointing to a role for NAD^+ capping in responding to this hormone.

And indeed, they found that the level of NAD^+ capping of RNA in response to abscisic acid dynamically changed.

"It does look like NAD^+ capping is tissue-specific and responds to at least one specific physiological cue," says Gregory, "at least in plants. That's pretty neat becaue it looks like it's a strong regulator of RNA stability, so the plant can destabilize different sets of mRNA transcripts, depending on where this process is acting and what cue is being given."

The group's findings even tied back to the unusual discovery they had made much earlier, of a build-up of small RNA molecules. In their DX01 mutant plants, they observed that the NAD^+ capped mRNA transcripts were processed into small RNAs, which are also unstable. Gregory, Yu, and colleagues believe this may be a secondary mechanism to remove NAD^+ and rid themselves of these noncanonically capped transcripts, even in the absence of DX01.

"What's going on is they're using another pathway, making small RNAs, perhaps to get back the NAD^+ so they can use it for other processes," Yu says.

Indeed, NAD^+ is a critical component in metabolism, so it makes sense that plants would have multiple strategies for ensuring they have enough available to them, the researchers say.

In future work, the Gregory lab hopes to continue exploring the NAD^+ mark, including working out how it is added and not just removed.

"Once we learn how to add, recognize, and remove it, it gives us the power to use this process as a tool for regulating various responses in plants," Gregory says, a power that could possibly be used in agriculture.

But human health could benefit from these insights as well. The Penn researchers say that the work deserves follow-up in mammalian systems. "I'd be curious to see what types of mRNA transcripts in mammals respond to different hormones," says Gregory.

Addition and removal of the NAD^+ cap may even be involved in cancer biology, Gregory and Yu say. The abnormal cell metabolism seen in cancer cells often owes to mishaps in the type of regulation that mRNA transcripts undergo, and there's a "real probability," Gregory says, that NAD^+ capping and decapping could play a role.

For his part, Gregory is pleased to have been able to move forward with an area of research that eluded him years ago, one that is opening up a new area of study for his lab.

"This is definitely one of those stories that reminds me that science is not a sprint; it's a marathon," Gregory says.

More information: Xiang Yu et al, Messenger RNA 5′ NAD+ Capping Is a Dynamic Regulatory Epitranscriptome Mark That Is Required for Proper Response to Abscisic Acid in Arabidopsis, *Developmental Cell* (2020). DOI: 10.1016/j.devcel.2020.11.009

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