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RESEARCH NEWS

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Enzyme is Crucial for Production of Plant Growth Hormone

Researchers have identified an enzyme involved in the production of auxin, a plant growth hormone that influences many aspects of plant growth, including cell division and flowering. Although auxin has been studied for more than 100 years, scientists have not had a good grasp of how the hormone is synthesized by plants.

In an article published in the January 12, 2001, issue of the journal *Science*, Howard Hughes Medical Institute investigator Joanne Chory and colleagues at the Human BioMolecular Research Institute in San Diego and the University of Minnesota reported identifying a new flavin monooxygenase (FMO)-like enzyme that is central to auxin biosynthesis. The finding reveals an important pathway for auxin synthesis and is likely to offer clues that will aid researchers studying similar enzymes in mammals. The role of the FMO-like enzymes was discovered when the scientists created a mutant form of the plant *Arabidopsis* that had growth characteristics indicative of auxin overproduction. *Arabidopsis*, a small flowering plant that is a relative of the mustard plant, is the basic model organism used in plant biology research.

"The finding reveals an important pathway for auxin synthesis."
— Joanne Chory

"We were randomly inserting into the *Arabidopsis* genome DNA sequences called enhancer sequences that promote gene activity," said Chory, who is at The Salk Institute for Biological Studies. "Specifically, we were looking for mutants in the light-responsive pathway. One measure of the light-responsive pathway in plants is the length of the primary stem, or hypocotyl, under various light conditions," Chory explained. "Normally, light represses stem growth so that the stem becomes thicker and can support more leaves. In plants that don’t respond well to light due to mutations, the hypocotyl elongates."

According to Chory, one mutant produced during their experiments showed signs of overgrowth that is characteristic of auxin overproduction. "This mutant had long hypocotyls and increased apical dominance with down-curling leaves," said Chory. Apical dominance is the inhibition of lateral branching characteristically produced by auxin.

"These characteristics resulted in a plant that resembled the yucca, so we named the mutant *yucca*. Importantly, all these characteristics were sort of shouting out to us that there was an overproduction of auxin," said Chory.

If the outward appearance of the *yucca* mutant proved to be caused by auxin overproduction, then the dominant, fertile *yucca* could offer the first experimental system in which to study how auxin is synthesized. Previous attempts by other researchers to produce loss-of-function auxin mutants—the standard approach to exploring a synthetic pathway—met with no success.

Analysis of the *yucca*-mutant plants revealed that they did, indeed, show increased auxin levels, said Chory. Also, physiological and genetic experiments on the plants showed that these high auxin levels caused the distinctive growth characteristics of the *yucca* mutant. Also, the scientists were able to repress *yucca*'s distinctive growth characteristics by using genetic techniques that specifically reduced auxin levels in the mutant plant.

To test whether the pathway controlled by the *yucca* gene was also likely to be used for auxin synthesis in other plants, the researchers overexpressed the *YUCCA* gene in tobacco plants. These experiments dramatically altered the tobacco plant, creating tobacco plants that resembled the *yucca*-induced changes in *Arabidopsis*.

To identify the enzyme expressed by the *YUCCA* gene, the scientists did genetic studies and found that the gene resembled FMO genes found in mammals, said Chory. Searches of the genome database of *Arabidopsis* revealed that the plant possessed two families of FMO-like genes. When the scientists used activation tagging to overexpress some of these genes in plants, the plants resembled the *yucca* mutant. Such redundancy, said Chory, finally explains why past efforts to produce knockout auxin mutants failed.

Plant biologists have debated whether auxin biosynthesis proceeded via a pathway that is dependent on the amino acid tryptophan. Studies of this FMO-like enzyme in *yucca* revealed that the enzyme most likely catalyzes the oxygenation of the compound tryptamine, a key finding, said Chory.

"That finding made us go back and look at the proposed pathways for auxin biosynthesis, none of which have been sorted out in the plant. And since the tryptamine that this enzyme acts on comes from the tryptophan-dependent pathway for auxin biosynthesis, this shows that in *yucca*, auxin biosynthesis proceeds via a tryptophan-dependent pathway."

However, she added, the picture of auxin biosynthesis is complicated by the fact that other scientists have produced mutant plants that cannot produce tryptophan, yet they can still produce auxin. "Thus, there is an alternative route, but at least now we can begin to propose a pathway for the tryptophan-dependent part of auxin biosynthesis."

Chory and her colleagues are now exploring the different FMO-like enzymes in *Arabidopsis*, and she believes that insights from the plant enzyme could yield insight into the function of the mammalian counterparts.

"No one knows the reason why animals have these enzymes," she says. "They know that the FMOs can act to detoxify xenobiotics—naturally-occurring toxic compounds in food. Our results suggest that researchers studying these mammalian enzymes should look at tryptophan metabolism as one of the important physiological roles for these enzymes."