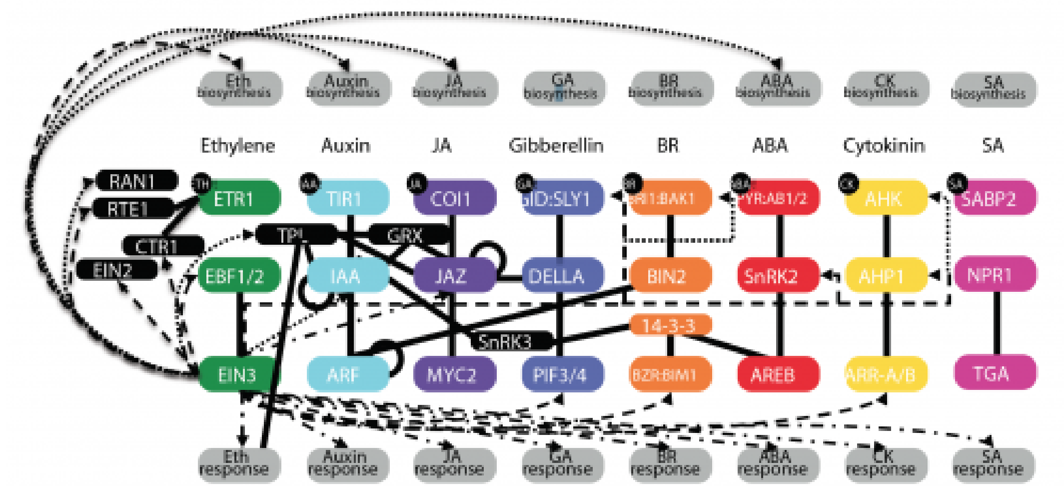
Scientists identify thousands of plant genes activated by ethylene gas

June 11 2013



The gaseous hormone ethylene, also known as the fruit ripening hormone, "talks to" many of the other plant growth controlling pathways using a protein called EIN3. The image displays gene networks for each of the major plant hormone biosynthesis, signaling and response pathways and which genes the EIN3 protein "touches" (potentially regulates). Credit: Courtesy of Katherine Chang, Salk Institute for Biological Studies

It's common wisdom that one rotten apple in a barrel spoils all the other apples, and that an apple ripens a green banana if they are put together in a paper bag. Ways to ripen, or spoil, fruit have been known for thousands of years—as the Bible can attest—but now the genes underlying these phenomena of nature have been revealed.

In the online journal *eLIFE*, a large international group of scientists, led by investigators at the Salk Institute for Biological Studies, have traced the thousands of genes in a plant that are activated once ethylene, a gas that acts as a plant growth hormone, is released.

This study, the first such comprehensive genomic analysis of ethylene's biological trigger, may lead to powerful practical applications, the researchers say. Ethylene not only helps ripen fruit, it also regulates growth and helps defends a plant against pathogens, among a variety of other functions.

Teasing out the specific genes that perform each of these discrete functions from the many genes found to be activated by ethylene might allow scientists to produce plant strains that slow down growth when needed, accelerate or prevent ripening, retard rotting or make plants more resistant to disease, says the senior investigator, Joseph R. Ecker, of Salk's Plant Molecular and Cellular Biology Laboratory.

"Now that we know the genes that ethylene ultimately activates, we will be able to identify the key genes and proteins involved in each of these branch pathways, and this might help us manipulate the discrete functions this hormone regulates," Ecker says.

By all accounts, it took a Herculean effort to decode the genetic pathways that ethylene activates—one that involved four institutions and 19 researchers, many of whom normally work in human biology. For example, Ecker invited the expertise of Carnegie Mellon University computer scientist Ziv Bar-Joseph, transcriptional expert Timothy Hughes from the University of Toronto, as well as computational biologist Trey Ideker and genomicist Bing Ren from the University of California, San Diego.

The study also represents a milestone for Ecker, who has devoted his career to understanding the power exerted by plant-based ethylene.

"I have been trying, for several decades, to understand how a simple gas—two carbons and four hydrogens—can cause such profound changes in a plant," Ecker says. "Now we can see that by altering the expression of one protein, ethylene produces cascading waves of gene activation that profoundly alters the biology of the plant."

Although the plant they studied is the *Arabidopsis thaliana*, related to cabbage and mustard, ethylene functions as a key hormone in all plants, he adds.

The researchers looked at what happens in *Arabidopsis* after ethylene gas causes activation of EIN3, a master transcription factor—a protein that controls gene expression—that Ecker had discovered and cloned in 1997. EIN3 and a related protein, EIL1, are required for the response to ethylene gas; without these proteins, ethylene has no effect on the plant.

"We wanted to know how ethylene is actually doing its job," Ecker says. "Once the plant responds to ethylene by activating EIN3, what happens? What genes are turned on? And what are those genes doing?"

Using a technique known as ChIP-Seq, the researchers exposed *Arabidopsis* to ethylene and identified all the regions of the plant genome that bound to EIN3, which required using next-generation sequencing. They then used genome-wide mRNA sequencing to identify those targeted genes whose expression actually changes due to interaction with EIN3. "Not all genes targeted by EIN3 have changes in their gene expression," Ecker says.

They found that thousands of genes in the plant responded to EIN3. Then the investigators discovered two interesting things. First, when EIN3 is activated by ethylene, it goes back to control the genes in the pathway that were used to activate the EIN3 transcription factor in the first place. "That tells us that a plant making a critical master regulator like EIN3 wants to keep that production pathway under very tight control," Ecker says. "We had not expected this, and now this gives us a strategy to understand genetic control of other plant hormones."

The second discovery is that EIN3 targets all other hormone signaling pathways in the plant. Ecker offers an analogy to understand the reasons why: "Imagine you are in a recording studio and you have one of those tables in front of you that have all of those switches. If you start pushing up the dials for one sound effect, you probably turn down the dial for other sound.

"If ethylene tells a plant to stop growing, it has to control other hormones that are telling the plant to grow," he says. "We found that about half of the genomic targets of the EIN3 protein are found in other hormone signaling pathways."

Control of those hormones by EIN3 is very complex and is accomplished in a 24-hour period during which four cascading waves of transcriptional regulation takes place, Ecker says.

In addition to gaining insight into how ethylene genetically controls diverse functions within a plant, he adds that findings from the study provides a template by which to decode the workings of other plant hormones—none of which have been as well studied as ethylene.

"Learning how plants coordinate hormone responses is essential to understanding their regulation of growth and development, be it in seed germination, fruit ripening, or responding to drought, insects, or pathogens," says Katherine Chang, the first author of the paper and researcher in Ecker's lab. "In this way, mapping interconnections between the hormone pathways may have implications in agriculture."

Provided by Salk Institute