Scientists find a molecular clue to the complex mystery of auxin signaling in plants

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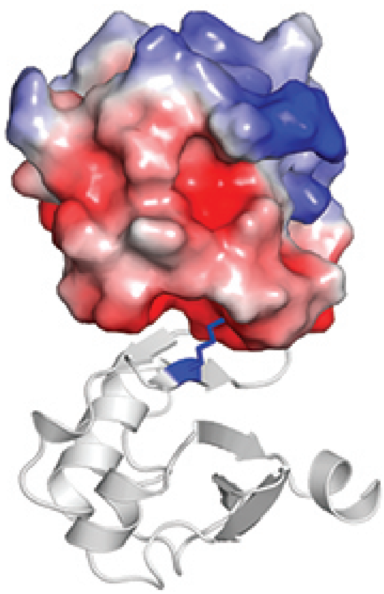


The transcription factor ARF7 turned out to have a magnet-like interaction region, called a PB1 domain, with positively (blue) and negatively (red) charged faces. Credit: Strader, Jez et al.

Wikipedia lists 65 adjectives that botanists use to describe the shapes of plant leaves. In English (rather than Latin) they mean the leaf is lance-shaped, spear-shaped, kidney-shaped, diamond shaped, arrow-head-shaped, egg-shaped, circular, spoon-shaped , heart-shaped, tear-drop-shaped or sickle-shaped—among other possibilities.

How does the plant "know" how to make these shapes? The answer is by controlling the distribution of a plant hormone called auxin, which determines the rate at which plant cells divide and lengthen.

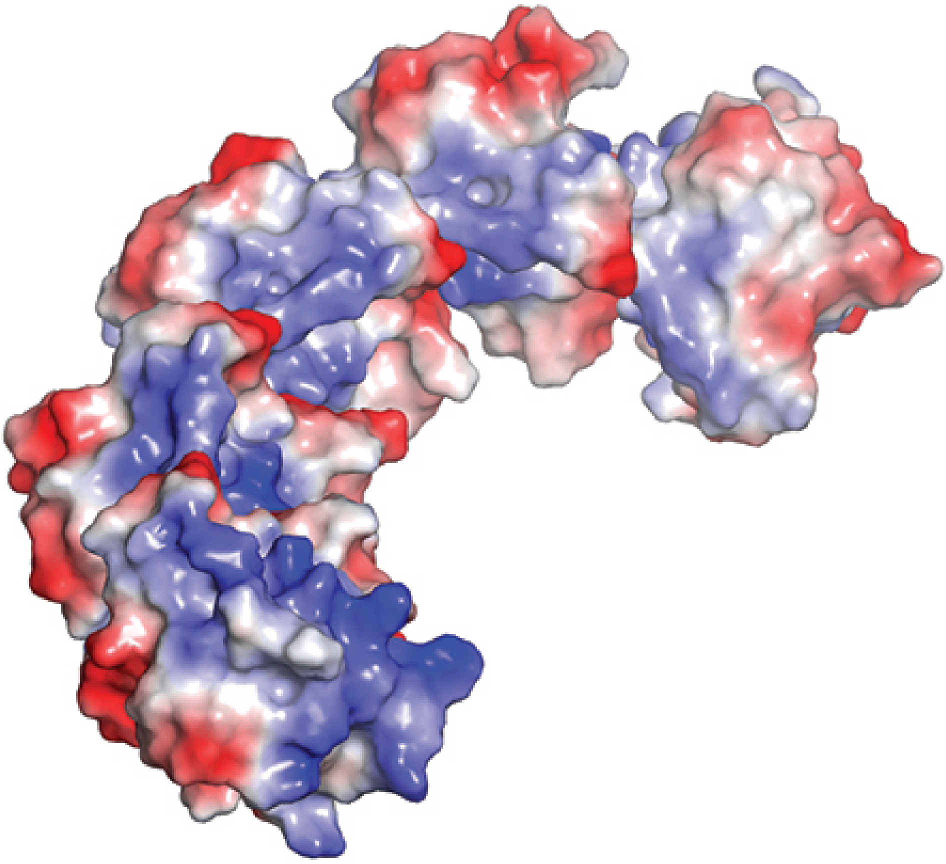
But how can one molecule make so many different patterns? Because the hormone's effects are mediated by the interplay between large families of proteins that either step on the gas or put on the brake when auxin is around.

In recent years as more and more of these proteins were discovered, the auxin signaling machinery began to seem baroque to the point of being unintelligible.

Now the Strader and Jez labs at Washington University in St. Louis have made a discovery about one of the proteins in the auxin signaling network that may prove key to understanding the entire network.

In the March 24 issue of the *Proceedings of the National Academy of Sciences* they explain that they were able to crystallize a key protein called a transcription factor and work out its structure. The interaction domain of the protein, they learned, folds into a flat paddle with a positively charged face and a negatively charged face. These faces allow the proteins to snap together like magnets, forming long chains, or oligomers.

We have some evidence that proteins chain in plant cells as well as in solution, said senior author Lucia Strader, PhD, assistant professor of biology and an auxin expert. By varying the length of these chains, plants may fine-tune the response of individual cells to auxin to produce detailed patterns such as the toothed lobes of the cilantro leaf.



The double-sided interaction domain may allow multi-protein chains to form. In Korasick's crystal, five of the ARF7 PB1 domains stuck to one another, forming a pentamer. "It was really beautiful to look at in the software, because you could actually see its spirals and turns," said Korasick. Credit: Strader, Jez et al.

Combinatorial explosion

Sculpting leaves is just one of many roles auxin plays in plants. Among other things the hormone helps make plants bend toward the light, roots grow down and shoots grow up, fruits develop and fruits fall off.

"The most potent form of the hormone is indole-3-acetic acid, abbreviated IAA, and my lab members joke that IAA really stands for Involved in Almost Everything," Strader said.

The backstory here is that whole families of proteins intervene between auxin and genes that respond to auxin by making proteins. In the model plant Aribidopsis thaliana these include 5 transcription factors that activate genes when auxin is present (called ARFs) and 29 repressor proteins that block the transcription factors by binding to them (Aux/IAA proteins). A third family marks repressors for destruction.

"Different combinations of these proteins are present in each cell," said Strader. "On top of that, some combinations interact more strongly than others and some of the transcription factors also interact with one another."

In an idle moment David Korasick, a graduate fellow in the Strader and Jez labs and first author on the *PNAS* article, did a back-of-the-envelope calculation to put a number on the complexity of the system they were trying to understand. From a strictly mathematical point of view there are 3,828 possible combinations of the auxin-related Arabidopsis proteins. That is assuming interactions involve only one of each type of protein; if multiples are possible, the number, of course, explodes.

To make any headway, Strader said, we had a better understanding of how these proteins interact. The rule in protein chemistry is the opposite of the one in design: instead of form following function, function follows form.

So to figure out a protein's form—the way it folds in space—they turned to the Jez lab, which specializes in protein crystallography, essentially a form of high-resolution microscopy that allows protein structures to be visualized at the atomic level.

Korasick had the job of crystallizing ARF7, a transcription factor that helps, Arabidopsis bend toward the light. With the help of Joseph Jez, PhD, associate professor of biology, Corey Westfall, and Soon Goo

Lee), Korasick cut "floppy bits" off the protein that might have made it hard to crystallize, leaving just the part of the protein where it interacts with repressor molecules.

After he had that construct, crystallization was remarkably fast. He set up his first drops in solution wells on the 4th of July. The protein crystallized with a fuss, and he ran the crystals up to the Advanced Photon Source at the Argonne National Laboratory outside Chicago. By August 1 he had the diffraction data he needed to solve the protein's structure.

Surprise, surprise

The previous model for the interaction between a repressor and a transcription factor – a model that had stood for 15 years, Strader said– was that the repressor lay flat on the transcription factor, two domains on the repressor matching up with the corresponding two domains on the transcription factor.

The structural model Korasick developed showed that the two domains fold together to form a single domain, called a PB1 domain. A PB1 domain is a protein interaction module that can be found in animals and fungi as well as plants.

Graduate student David Korasick commuted between the Strader Lab, which specializes in genetics, and the Jez Lab, which has expertise in structural biology, to learn how plants control the effects of the master hormone auxin. Credit: Strader Lab

Strader, Jez et al.

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The repressor proteins, which are predicted to have PB1 domains identical to that of the ARF transcription factor, then stick to one or the other side of the transcription factor's PB1 domain, preventing it from doing its job. Experiments showed that there had to be a repressor protein stuck to both faces of the transcription factor's PB1 domain to repress the activity of auxin.

This means the model, which pairs a single repressor protein with a single transcription factor, is wrong, Strader said.

"Nor can we limit the interactions to just two," she said. "It could be hundreds for all we know."

In Korasick's crystal five of the ARF7 PB1 domains stuck to one another, forming a pentamer.

"I like to think of the PB1 domains as magnets, " Strader said. "Like magnets, they can stick together, back-to-front, to form long chains."

"But we have to put an asterisk next to that," Korasick said, "because it's possible it's an artifact of crystallography and doesn't work that way in living plants."

But both Strader and Korasick suspect that it does. Strader points out that the complexity of the auxin signaling system has increased over evolutionary time as plants became fancier. A simple plant like the moss Physcomitrella patens has fewer signaling proteins than a complicated plant like soybean.

"Probably what that's saying is that it's really, really important for a plant to be able to modulate auxin signaling, to have the right amount in each cell, to balance positive and negative growth," Korasick said.

"The difference between plants and animals," said Strader, "is that plants have rigid cell walls. So when a plant cell decides to divide itself or length itself, that's a permanent decision, which is why it's so tightly controlled."

More information: Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression, www.pnas.org/cgi/doi/10.1073/pnas.1400074111

Provided by Washington University in St. Louis