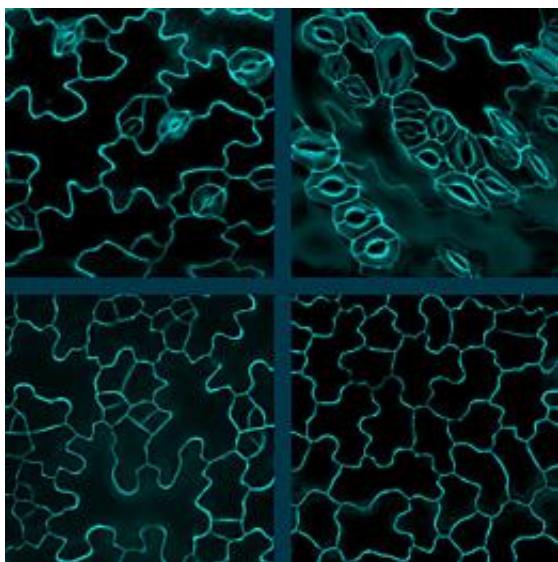


# Chemical signal helps plants control their “breathing”

January 13 2012, By Keiko U. Torii



Stomata are normally evenly spaced for photosynthetic gas exchange, as in the normal *Arabidopsis* seedling shown at the top left. In a plant lacking all ERECTA-family receptor kinases (top right), excessive stomatal clusters result. Treating normal seedlings with EPF1 (bottom right) or EPF2 (bottom left) alters stomatal development.

For most plants, staying alive means adapting quickly to a constantly changing environment. In a drought, staving off water loss is vital. On a sunny day, absorbing carbon dioxide to generate energy through photosynthesis is key. Now, researchers have discovered how plants regulate the development of stomata, the pores through which these critical exchanges with the environment occur.

“Stomata are really vital for plant growth and survival,” says Keiko Torii, a Howard Hughes Medical Institute - Gordon and Betty Moore Foundation investigator at the University of Washington who led the new work, which was published online January 12, 2012, in the journal *Genes & Development*.

“It will be interesting to study this in crop plants and see the relationship is between stomatal density and plant productivity,” said Keiko U. Torii

When their stomata are open, plants can absorb carbon dioxide and oxygen from the air and release oxygen, the byproduct of energy-generating photosynthesis. But open stomata also allow water to escape from the plant. So most plants open stomata during the day to allow photosynthesis to occur and close stomata during the night to prevent water loss.

Stomata function best when they are evenly spaced over a leaf's surface, says Torii, whose lab focuses on uncovering the genetics that underlie plant development. Different plants have different patterns of spacing, and until now, scientists have not understood how this pattern is established during development.

Torii and her colleagues knew that two related proteins, called epidermal patterning factor 1 and 2 (EPF1 and EPF2), control the fate of cells in developing leaves. Cells that are becoming stomata produce EPF2 as a signal that prevents other stomata from developing nearby. If high levels of EPF2 are applied to a developing plant, no stomatal precursors form. EPF1, in contrast, is required for final stomatal formation. If it's applied to a developing plant, stomatal precursors form but never fully differentiate into stomata.

Scientists have predicted that EPF1 and 2 interact with a handful of receptors—some in the ERECTA family of receptors, and another called

too many mouths (TMM). However, it was not known which receptors helped mediate each function, or even whether they truly interact directly. A cross-disciplinary collaboration between Torii and nanobiomaterials engineers Mehmet Sarikaya and Candan Tamerler, both professors at the University of Washington Materials Science and Engineering Department, led to a breakthrough. Sarikaya and Tamerler developed biosensor chips that enabled the team to quantitatively measure molecules binding to the receptors, and the kinetics of that interaction.

EPF1 and 2 are relatively large peptides with complicated folding patterns. This makes them hard for scientists to isolate and study. So Torii's team turned instead to the suspected receptors to study their effect on plants and narrow down how they might interact with the EPF proteins. But it still wasn't straightforward.

"We didn't want to just knock out receptors to study their functions, because they likely had overlapping functions. If we knocked out one receptor, another receptor would just take over the function," explains Torii. So they genetically engineered plants in a way that allowed them to block the downstream effects of any receptor.

When the effects of one receptor—ERECTA—were blocked, Torii's lab found that plants could no longer respond to EPF2 peptides to inhibit formation of stomatal precursors. They went on to show that EPF2 binds to, and acts through, this receptor. When the effects of another receptor—ERL1—were blocked, plants could no longer respond to EPF1 peptide to suppress differentiation of stomata. EPF1, they found, acts through ERL1. They also discovered how TMM binds to the ERECTA-family receptors to modulate their function. But TMM, they showed, limited binding to EPF peptides.

"It was a struggle to show all this and find a way to study these

interactions,” says Torii. “But now, going forward, we can start addressing the dynamics of these receptors in real time. We can take advantage of our cross-disciplinary technology to answer more questions.”

The team has only studied the ligand-receptor pairs in the model plant *Arabidopsis thaliana*, but wants to look into whether other plants use the same receptor sets to control stomata spacing.

“Some of our mutants that have fewer stomata still grow just fine under normal conditions and they lose much less water,” says Torii. “So it will be interesting to study this in [crop plants](#) and see the relationship is between stomatal density and plant productivity.” Tweaking the density of a crop plant’s [stomata](#) could offer farmers a way to save water, she says.

Provided by Howard Hughes Medical Institute

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