Press Release

Unlocking the mystery on how plant leaves grow their teeth

Discovery of EPFL2 peptide (key) and its receptor (lock) that make zigzag edges on leaves

September 2, 2016

Plant biologists at ITbM, Nagoya University have discovered the key element, an EPFL2 peptide that is responsible for creating the teeth-like shapes on plant leaves. The zigzag edges of leaves, so-called leaf teeth, are important for making the characteristic shapes of each leaf. This study illustrates the unexplored mechanism of leaf teeth formation and will shed light on finding out how leaves have developed to become the shapes that they are today.

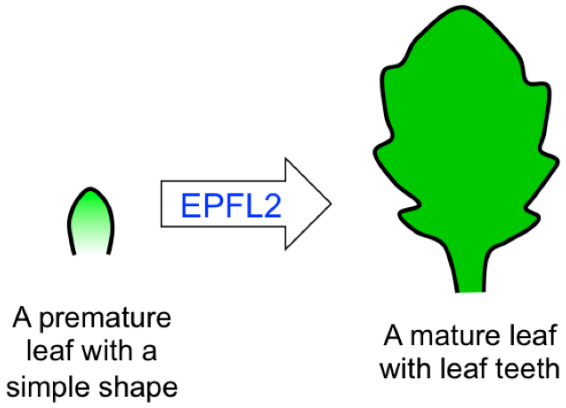


Figure 1. EPFL2, a peptide compound that makes plant leaves jagged.

Nagoya, Japan – Dr. Toshiaki Tameshige, Associate Professor Naoyuki Uchida and Professor Keiko Torii of the Institute of Transformative Bio-Molecules (ITbM) of Nagoya University, and their colleagues at the University of Washington (USA) and Nara Institute of Science and Technology (Japan) have reported their new findings in the journal *Current Biology*, on how a peptide and its receptors work to regulate auxin response and control leaf tooth growth in plants.

The plant hormone, auxin has been known to take part in the development of leaf teeth, but the exact mechanism of their formation has been a mystery up till now. In this study, the research group has found that a peptide called **E**PIDERMAL **P**ATTERNING **F**ACTOR-**L**IKE **2** (EPFL2) and its receptor protein, ERECTA family receptor kinases, control the amount of auxin during leaf tooth growth. In plant leaves where the EPFL2 peptide is inactive, the leaf becomes round without teeth.

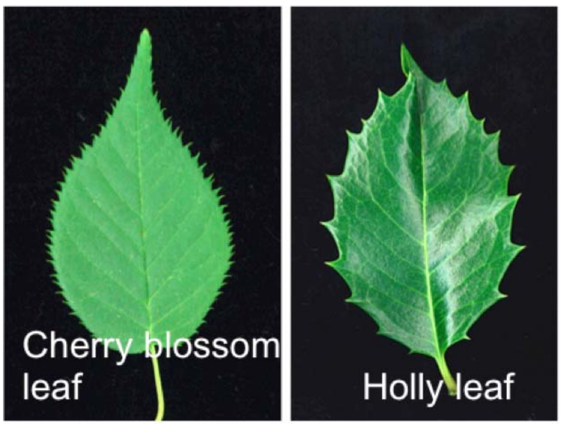


Figure 2. Leaves of cherry blossom and holly

Many leaves have small zigzags called leaf teeth on their margin. Examples of plants that contain leaf teeth include cherry blossoms, maples, dandelions and shiso that are used with many Japanese dishes. The leaf teeth of holly and thistle leaves have developed to become hard thorns to protect the plants themselves. Because of the pain that they cause, these leaves have been used as a charm against evil spirits or have appeared in myths to resemble sorrow and pain. Although plant teeth have been known to function for some plants as a means for protection, water drainage, and photosynthesis, the exact reason why they exist and how they are made are yet to be revealed.

In this study, the team has discovered the main substance that creates the plant teeth and found out how they work. The key substance that they discovered was a peptide called EPFL2.

"I first started to wonder about the shape of leaves in 2012," says Uchida, one of the authors of this study. "I originally started this work with Satoshi Okamoto, a student in our group." Toshiaki Tameshige, a postdoctoral researcher at ITbM joined the team in 2013 to take up this work based on his expertise in plant leaves.

"My interest in plant leaves dates back to ten years ago, in 2006," says Tameshige, who conducted the experiments. "I was attracted to leaves because of its beautiful appearance and was fascinated by their various shapes. We decided to look into the function of EPFL2 to see its effect on leaf shapes."

EPFL2 is a peptide (a chain of amino acids) that has recently been discovered. Like the plant hormone, auxin, EPFL2 is secreted from plants but its function had not been uncovered yet. By using the model plant, *Arabidopsis thaliana*, the team studied the function of EPFL2 and found that plants that are unable to synthesize EPFL2 grow with rounded leaves, which do not develop the leaf teeth that are usually present in the wild type. Thus, they found that EPFL2 was essential for the formation of leaves with a zigzag edge.

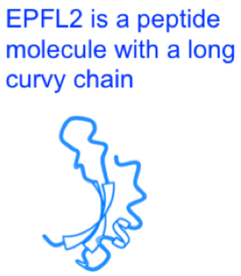


Figure 3. (left) Structure of EPFL2.

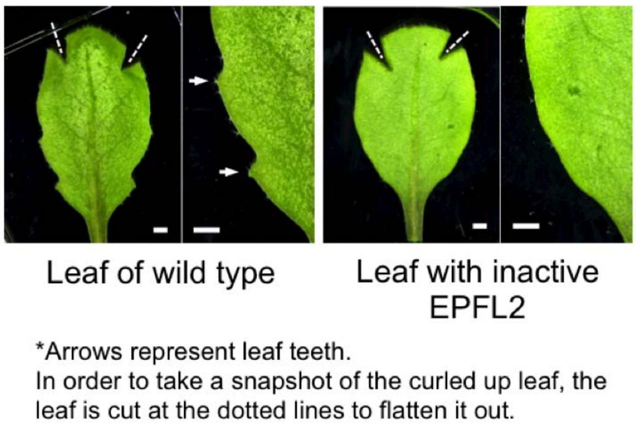
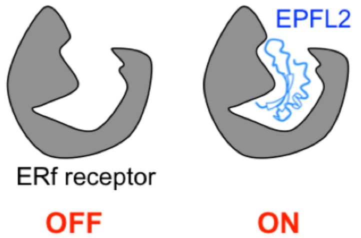


Figure 4. (right) Leaves of wild type and those with inactive EPFL2.

Compounds such as the EPFL2 peptide and auxin, which control plant growth, act as 'keys' to activate specific physiological processes. The keys work by interacting with a 'lock', in other words, a receptor protein that binds with plant growth substances. In this study, the group succeeded in identifying the EPFL2 receptor along with EPFL2 for the first time.



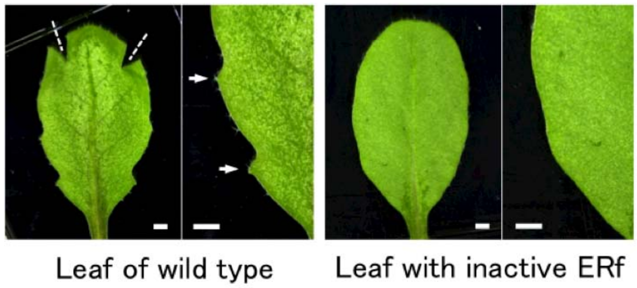
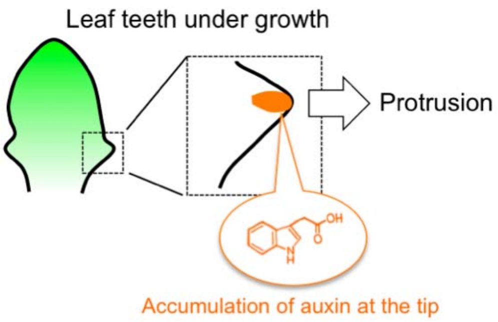


Figure 5. (left) Binding of EPFL2 to its receptor. **Figure 6. (right)** Leaves of wild type and those with inactive ERf.

The receptor of the EPFL2 peptide was characterized as a protein, which is part of the **ER**ECTA **f**amily (ERf). The group found that the plants, which have lost some of the functions of the ERf receptor have developed leaves without teeth in a similar manner to the plants that are unable to make EPFL2.

"The most difficult part of this research was quantifying the degree of zigzags for leaf teeth," says Tameshige. "I have tried several different calculation methods and managed to develop a method for quantifying and comparing leaf teeth. After looking at over 1000 leaves, I was pleased to find evidence that EPFL2 plays an essential role in creating zigzag edges on the leaves."

For the leaf to gain its shape, the leaf starts from a small round shape and growth in specific parts of the leaf are controlled as the plant grows. In other words, growth in some parts of the leaf is enhanced whereas growth in other parts is suppressed to create protrusions and dents in leaves, respectively. There have been reports that auxin is responsible for the edges to protrude and grow into leaf teeth. Auxin is known to accumulate at the tips of leaf protrusions for plants under growth, and does not accumulate in the surrounding skirts of the tip. This difference in the concentration of auxin is necessary for the development of leaf protrusions, but the origin of this concentration difference during tooth growth has been unexplained up till now.



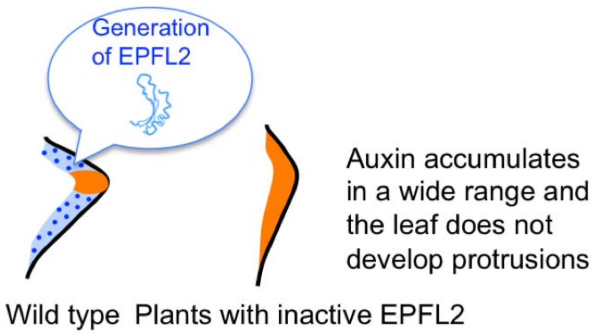


Figure 7. (left) Formation of a protrusion by auxin. **Figure 8. (right)** Leaves of wild type and those with inactive EPFL2.

By studying the relationship between the EPFL2 peptide and auxin, the group found that EPFL2 inhibits the accumulation of auxin at skirts of tooth tips. Since EPFL2 was not synthesized at the tip of the leaf teeth but was only present at the skirts of the teeth, this prevented the accumulation of auxin at the skirts of the tip. In plants that are unable to make EPFL2, auxin spreads across the margin of the leaf and thus, leaf teeth are not generated due to the absence of different auxin concentrations across different regions.

The team next studied why the EPFL2 peptide was not made at the tip of the leaf teeth, but was only present at the skirts of the tips. Interestingly, they found evidence indicating that auxin determines the position of EPFL2 generation, i.e. where auxin accumulates, EPFL2 is not synthesized at that position. Therefore, the position where auxin accumulates and the position where EPFL2 is being generated resemble a photographic relationship of positive and negative images.

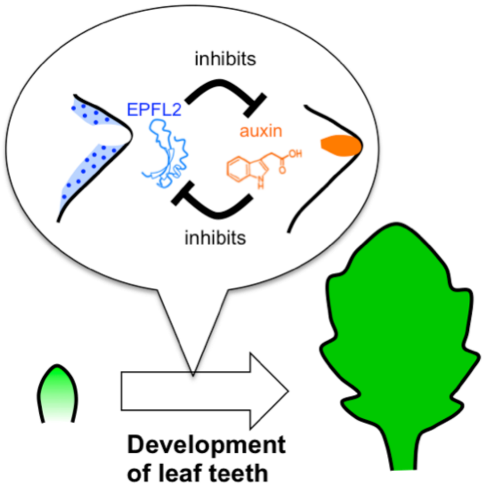


Figure 9. Feedback loop by EPFL2 and auxin

"I was really excited the moment when we saw that EPFL2 and auxin were in an inverse relationship," says Uchida. "It is difficult to say which is first, as it is like the chicken or the egg; whether auxin determines its accumulation position first or EPFL2 determines where it is synthesized first." This type of mutual relationship, where two substances inhibit each other is called feedback control and is a common mechanism found in various processes, such as shaping of the body and the circadian clock.

"This mechanism on how the EPFL2 peptide creates leaf teeth was uncovered using *Arabidopsis thaliana*," explains Tameshige. "We hope to see whether the same mechanism occurs in other plants, but our results suggest that EPFL2 may be responsible for creating leaves that have more zigzag edges or have prickly thorns," he continues.

"We are also interested in investigating whether we can synthetically prepare the EPFL2 peptide and use it as an additive to create leaves with unique shapes, for example in foliage plants and in bonsai," says Uchida.

"Furthermore, we may be able to change the leaf shape of vegetables, which will change its appearance as well as its food texture," says Tameshige. "If we can change the shape of leaf vegetables such as lettuce and spinach, this may lead to the generation of new species or brand vegetables that have premium value."

"My next goal is to be able to predict and reproduce the shape of leaves using computer simulation," says Tameshige. "I hope to make mathematical models to explain the relationship between EPFL2 and zigzag edges of leaves. It would be interesting to be able to design plants at will using computer modeling."

This article "A secreted peptide and its receptors shape the auxin response pattern and leaf margin morphogenesis" by Toshiaki Tameshige, Satoshi Okamoto, Jin Suk Lee, Mitsuhiro Aida, Masao Tasaka, Keiko U. Torii and Naoyuki Uchida, is published online on September 2, 2016 in *Current Biology*. DOI: 10.1016/j.cub.2016.07.014 (http://dx.doi.org/10.1016/j.cub.2016.07.014)

From the left: Professor Keiko Torii, Associate Professor Naoyuki Uchida, Dr. Toshiaki Tameshige

About WPI-ITbM (http://www.itbm.nagoya-u.ac.jp/)

The Institute of Transformative Bio-Molecules (ITbM) at Nagoya University in Japan is committed to advance the integration of synthetic chemistry, plant/animal biology and theoretical science, all of which are traditionally strong fields in the university. ITbM is one of the research centers of the Japanese MEXT (Ministry of Education, Culture, Sports, Science and Technology) program, the World Premier International Research Center Initiative (WPI). The aim of ITbM is to develop transformative bio-molecules, innovative functional molecules capable of bringing about fundamental change to biological science and technology. Research at ITbM is carried out in a "Mix-Lab" style, where international young researchers from various fields work together side-by-side in the same lab, enabling interdisciplinary interaction. Through these endeavors, ITbM will create "transformative bio-molecules" that will dramatically change the way of research in chemistry, biology and other related fields to solve urgent problems, such as environmental issues, food production and medical technology that have a significant impact on the society.

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