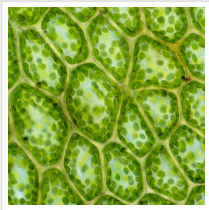
Improving Crop Resilience with Nanoparticles



2022-08-13
Materials that can carry CRISPR gene-editing into plant cells could be key in the fight against global hunger. A method for allowing scientists to see whether a nanoparticle will enter chloroplasts in plant cells will advance gene-editing techniques.

There were sceptics when Michael Strano and his colleagues published their method for using nanoparticles to alter the biology of living plants. In a letter to Nature Materials, one prominent plant scientist stated that the findings were wrong. “She wrote to the editor and said, ‘What these authors are proposing is not possible. We think they’re misinterpreting their data’,” Strano recalls.

But the chemical engineer at the Massachusetts Institute of Technology (MIT), in Cambridge, won over his critics, overturning an assumption that the membrane of the chloroplast — an organelle within plant cells that is responsible for photosynthesis — was impervious. “We had real-time video of particles going into this seemingly impenetrable chloroplast,” he says. The method, known as lipid exchange envelope penetration (LEEP), allows scientists to calculate where a nanoparticle will go to inside a cell — such as into the chloroplast or another organelle — or whether it will remain in the cytosol, the fluid that surrounds the organelles. This information can inform the design of nanoparticles that carry gene-editing machinery to targeted areas to rewrite the plant’s genome and imbue it with properties such as pest and disease resistance.

In particular, researchers are exploiting the CRISPR gene-editing system to engineer food crops that offer higher yields, or plants that produce compounds used in medications. The technology, for which Jennifer Doudna and Emmanuelle Charpentier shared the 2020 Nobel Prize in Chemistry, allows specific stretches of DNA to be targeted for editing, deletion or replacement.

The CRISPR-mediated editing of plants is already paying off. In 2021, the first CRISPR-edited food was introduced to the market, a tomato called Sicilian Rouge. The fruit produces high amounts of γ-aminobutyric acid, which its makers say can help to lower blood pressure. Teams in South Korea and Vietnam, meanwhile, have used CRISPR to make a tomato that flourishes in high salinity. To speed up such research, and to deliver CRISPR and their associated proteins (Cas) to a target location in the cell, scientists are exploring how nanoparticles can be used as carriers for genetic material.

The challenge is getting CRISPR–Cas into the cell nucleus. Plants don't have the same kind of circulatory system as animals, which means scientists cannot simply inject material into a plant and predict its trajectory. Furthermore, each plant cell is surrounded by a thicker and stronger wall than those in animal cells. The main barrier protecting the cells is a lipid membrane comprising two layers of fatty molecules, with a hydrophobic and a hydrophilic side, to keep foreign material out. The chloroplast inside the cells — which has a distinct genome from the rest of the plant — is protected by a double membrane that CRISPR must penetrate.

LEEP helps by delivering carbon nanotubes (tiny cylinders made of a single rolled layer of carbon atoms) to plant cells. The nanotubes are wrapped in a layer of polymer, which also has hydrophobic or hydrophilic surface areas, and can cross cell membranes. With the right charge, the polymer combines with the plant's lipid membrane, carrying the nanotube with it. It's easy enough to attach genetic material to the nanotube, so the whole system gets carried through the chloroplast's double membrane, delivering DNA or RNA to its desired location.

LEEP has broad-ranging applications, says Strano, as it can predict which particles go into a plant cell and the chloroplasts. "It works for almost all nanoparticles — carbon, gold, silica," he says. It also has an advantage over other methods in that it should work for many species of plants. Often geneticists use a model crop, such as tobacco, to test their theories, but struggle to translate the results more widely. "The criticism has always been, 'You can do this fancy technique in tobacco, but what about important plants like rice, wheat and maize?'" says Strano, who has tested LEEP in a dozen species, including spinach, rocket and watercress.

Strano is in a good position to continue testing LEEP. MIT is the leading US institution in nanoscience and nanotechnology-related output in the Nature Index, which tracks articles in 82 selected natural-sciences journals. Globally, the institution is placed 13th (see page S22). China, whose institutions dominate the top 10, overtook the United States as the leading country in the field in 2018, a position it retains.

A tool for wide use

Feng Wang, a biomedical engineer who directs the Nano Bio Interface Lab at Hefei University of Technology in Anhui, China, says LEEP is a breakthrough that allows particles to be customized to accommodate different payloads. CRISPR editing elements, such as plasmids (a circle of DNA) and mRNA, have different molecular sizes and surface charges, he says, "causing the surface of nanomaterials to be more complex and making entering into the plant cells more uncertain". Wang says researchers need to explore whether, and how well, LEEP can deliver CRISPR for gene editing.

There are competing methods for delivering genetic material to plant cells, but they all have limitations. One is the gene gun. Bits of DNA are dehydrated and attached to gold nanoparticles, which are then fired at the cells. This 'biolistic' approach inserts DNA deep into the cells, but can cause tissue damage and might deposit DNA in the wrong place. Electroporation, which uses a pulsed electrical field to change the permeability of the membrane, is sometimes used in animals, but can cause irreversible damage in plants. Agrobacterium tumefaciens, an infectious agent, can carry the genetic cargo, but only works on specific species, and if genes from the bacterium are inadvertently incorporated into the plant, they have to be bred back out while retaining the CRISPR-induced mutation. Scientists would like a more versatile tool.

Nanomaterials, including carbon dots, gold nanorods and magnetic nanoparticles, are attractive for plant genome editing, says Wang, because they can cost hundreds of times less than electroporation or gene guns, and are relatively easy to use.

Sizing up the situation

There are many questions about the use of nanomaterials for delivery of CRISPR, says Markita Landry, a chemical engineer at the University of California, Berkeley. One is the optimal size of the nanoparticle, which needs to be small enough to enter the cells and not get stuck, but large enough to attach its CRISPR–Cas cargo to. “Cas is a big protein, which is one of the challenges in its delivery, so one workaround is to code for it in a DNA plasmid,” Landry says. The plasmid carries the code that instructs the cell to make its own CRISPR molecule, and that new molecule then performs the editing (much like how mRNA COVID-19 vaccines instruct cells to make the spike protein that triggers the immune system).

Another consideration is making sure the cargo detaches from the nanoparticle at the right time. In one scenario, the cargo may not need to be released; the nanoparticle could travel to the cell’s nucleus, where DNA encoding CRISPR–Cas components, still attached, can interact with the plant’s DNA. In other applications, which Landry and her group are exploring, the particle may embed itself in the cell wall and release its cargo into the cell.

It’s also important to use methods that ensure CRISPR cuts the genome in the right area. Plant genomes are often larger and more redundant than that of humans, and the risk of off-target editing is high. “You think of how complicated a human is — these plants are orders of magnitude more complicated,” says Landry.

Read the original article on Nature.