Plant Synthetic Biology: A Budding Field

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Plant synthetic biology has its roots in agriculture — prehistoric farmers first genetically modified crops through the process of selective breeding. Today, plant synthetic biology involves transforming natural plant systems and pathways into new, synthetic systems that can increase the quantity and quality of biological products.

The fields's chief concern is still improving agriculture, but optimization of photosynthesis is also a popular topic of research. Recently, scientists successfully manipulated this metabolic pathway to improve carbon fixation by plants.

Over the past decade, researchers have also achieved a greater understanding of the functions of promoters and transcription factors in plants and how they can be modified to fit our needs.

However, plants can be difficult to engineer — their genetic components, including their promoter systems, are generally more complex than those of prokaryotic and some eukaryotic organisms. Consequently, synthetic biologists have made more headway in genetically modifying prokaryotic microbes.

Every organism has a genome, or a set of instructions that tell its cells how to eat, how to communicate, and more. The genome is programmed into every cell. However, at any given moment, only a subset of the instructions dictated by the genome are being followed by a specific cell type; for example, liver cells do not need to also be skin cells.

The way that cells read and implement certain parts of the genome are referred to as transcriptional programs — DNA instructions are transcribed into RNA, and then into functional proteins such as transcription factors, which turn on a particular gene that controls a desired function or phenotype.

Targeting Plant Genomes

Targeting the plant genome to selectively express desirable genes, such as larger leaves or more of a chemical compound, is both time- and labor-intensive. Arjun Khakar, a researcher at the Univ. of Minnesota (UMN), spent most of his time as a graduate student attempting to minimize branching in plants through genetic modification. Designing his novel transcription factor and inserting it into the plant genome took about a month — but obtaining the results took more than two years.

Because plants are multicellular organisms, unlike easily engineered, unicellular microbes, it is impossible to edit every cell in a full-grown plant. Scientists must modify the gene during the plant's single-cell stage, or right at formation. The effects of this change, however, are not visible until after at least three generations of plant growth. Changes in plant DNA take several generations to manifest.

"It takes a really long time for cells to grow into their final form, and in the case of plants, it can be several years," says Khakar. "That really slows down our capacity to test modifications in plants. It's one of the major reasons plant synthetic biology specifically has been growing so slowly — we're chained to this idea that we have to integrate constructs into the plant genome, and then wait for the lifecycles of the plant to propagate that change. Our design is limited and slowed by the life cycle."

At UMN, researchers are using a unique technique to modify plant genomes more quickly and broadly than traditional methods. They are targeting transcriptional programs and attempting to manipulate them to turn on or off certain genes — specifically, the genes that control leaf and stem shape and size in tomato plants. One of their goals is to make a tomato plant with longer stems and smaller leaves.

To circumvent the long testing periods typical of plant synthetic biology, they are using RNA vectors with targeted transcriptional factors, which induce expression of specific promoter regions on the desirable genes. Thus, changes in gene expression are visible in just weeks, rather than years.

"Rather than integrating modifications into the entire plant genome, which takes time, we're simply adding the secondary set of specific instructions, carried by RNA," says Khakar. "But we also needed to make sure that the RNA was self-sustaining, meaning that it could replicate itself without relying on DNA-to-RNA transcription. In addition, we needed to ensure that the RNA vector could move from plant cell to plant cell, delivering instructions throughout the organism."

The engineered RNA vectors rely on RNA polymerase, a protein that uses existing RNA as a template to create more RNA — mimicking self-replicating RNA viruses such as Ebola. Thus, the RNA is maintained over months, enabling the delivery of RNA instructions to plant tissue over its entire lifetime.

However, inserting RNA vectors into the plant targets only 10% of the cells in the vicinity of the injection, and the researchers wanted to transform the entire plant with their synthetic RNA.

They decided to manipulate the plant's plasmodesmata, *i.e.*, the series of channels that link plant cells to each other and can transport proteins throughout the plants. RNA vectors injected into the leaves moved through the plant via the channels, effecting change in each part of the plant.

To test their technology, the scientists synthesized an RNA vector that contained a synthetic transcription factor. The construct targets the specific promoter region on the tomato plant gene that controls leaf and stem shape and size.

After the RNA replicated within the plant for 2–3 weeks, they were able to measure strong gene expression in the plant for smaller leaves and longer stems.

The UMN researchers hope to find other applications of their technique, such as creating sturdy, renewable materials from bamboo. According to Khakar, plant synthetic biology will play a huge role in protecting crops from the effects of climate change, and could also help improve our environment through carbon sequestration. There is a great deal of growth in the field, he says.

This RNA vector technology is unique because it could have broad applications. Only a few plant varieties can be transformed using traditional gene editing techniques. The RNA vectors have a wider host range — the UMN researchers began testing in tomato and rice plants, but quickly moved to petunia and bamboo.

Growing a knowledge base

If plant synthetic biology expands to other species, it is essential that scientists are knowledgeable about multiple plant pathways and genomes.

At Oregon State Univ. (OSU), plant synthetic biologists have developed the Gramene database (www.gramene.org), an information source that holds genome, gene expression, pathway, and phenotype information for 67 plant species.

"We are trying to create a base built on the knowledge accumulated in literature over time," says Sushma Naithani, a researcher at OSU. "Our database is a major resource for plant synthetic biologists to provide a conceptual framework for a plant genome, and consequently build a network of proteins to yield a metabolic resource. Scientists can use the source to identify genes and pathways, and formulate more data-driven hypotheses."

For example, scientists working on improving the cultivation of several different strains of rice can use the website to analyze and compare changes between the species.

They can also identify single-nucleotide polymorphisms (SNPs), *i.e.,* subtle changes in the rice strains. The database helps researchers determine how these mutations affect protein expression, promoter activity, and more, in order to identify the consequences of genetic variations on a transcriptional level.

Gramene is a free database that was founded almost 20 years ago and is growing every year. "It's a basic resource for synthetic biologists to identify and compare information," says Naithani. "In synthetic biology, either you're correcting the mutation-blocking function of a gene, or adding something to enhance the function and quantity of a gene by tweaking it. This reference will help scientists identify those targets."

Examining Interactions

Often, scientists and engineers shift their focus away from the plant, and towards the soil and microbial environment around the plant root. The rhizosphere is an area of the soil found around the roots of a plant, which harbors microbes that are vital in influencing factors such as crop yield and production.

Dr. Yang Bai at the Institute of Genetics and Developmental Biology at the Chinese Academy of Sciences studies the link between the root microbiota and the its effect on plants. For instance, the team studied various compositions of microbiota in the rhizosphere for soybean plants, and studying the symbiotic efficiency of the system[i]. The ability to engineer the microbiota to influence plant growth can be significantly advantageous for farmers.

Similarly, Dr. Barney Geddes and his team at Oxford University have also been engineering the rhizosphere microbiota to improve crop yields, even in nutrient-poor soils. The team accomplished this by engineering cereal plants to produce chemical signals to control root bacteria, allowing them to take advantage of growth-promoting services of bacteria[ii]. The ability to produce greater crop yields in the presence of nutrient-poor soil can lead to significant advances in agriculture and developing energy from biomass.

Creating novel bioproducts

Once scientists have uncovered fundamental knowledge relating to factors such as gene expression and metabolic pathways, the information can be utilized to create products that have added value, or produce existing products in a more environmentally efficient manner.

At the Rothamsted Research, Dr. Peter Eastmond leads a team of scientists and engineers working on engineering plants to produce oils with a chemical structure that mimics human milk.

Human milk contains a large amount of triacylglycerol and a unique arrangement of fatty acids, which are thought to have unique benefits to human health yet are not abundantly present in infant formulas[iii]. By engineering a plant's metabolic pathways to produce these lipids, the team offers an alternative method for manufacturing formula that could provide additional health benefits.

Aside from food products, plant metabolic pathways can also be engineered to produce compounds that can be used as fuels or used to construct new materials. These manufacturing methods can be more environmentally friendly, but may take more research before such methods are implemented on an industrial scale.

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This article is based on presentations given at AIChE's 3rd International Conference on Plant Synthetic Biology, Bioengineering, and Biotechnology, Cambridge, U.K. (Oct. 4–6, 2019). The conference brought together scientists and engineers from universities, industry and government working in all aspects of Plant Synthetic Biology, Plant Bioengineering and Plant Biotech, and featured 7 sessions: (1) Engineering Plat Development and Signaling; (2) Food and Nutritional Security; (3) Photosynthetic Factories; (4) New Tools and Technologies in Synthetic

Biology; (5) Novel Bioproducts; (6) Engineering Biotic Interactions; (7) Engineering Primary Productivity.

Because of the success of the conference, the Society for Biological Engineering (SBE) will host the 4th International Conference on Plant Synthetic Biology, Bioengineering, and Biotechnology on October 30-November1, 2020 on a virtual platform. Please visit aiche.org/plantsynbio to submit an abstract, and apply for funding to attend.

References

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