Programming plants

Katie Cottingham

Computers have revolutionized how people do business, interact with friends, and find information. With the right inputs, computers churn out loads of information or, with artificial intelligence, they can create brand-new stories, poems, or images. The possibilities seem endless.

- **Synthetic biology enables researchers to program organisms, just as computer scientists program computers.**
- **Researchers at Stanford University are using synthetic biology tools to program plants to use water more efficiently.**
- **Plants that use water more efficiently could better withstand climate change.**

These possibilities are what attracted Jennifer Brophy and Janina Tamborski of Stanford University to synthetic biology, a branch of science that uses computer engineering concepts to redesign organisms—plants, in this case—with desired properties. With this framework, they constructed modules of genetic material that they transferred into plants to produce certain expression patterns.

"What is really exciting about this way of engineering plants is that we can make really precise, targeted and quantitative changes that we have not been able to do before," says Tamborski, a postdoc in Brophy's lab.

Brophy and Tamborski, along with their collaborators, have developed many modules and tools based on computer programming logic and are using them to better equip agricultural crops to endure a changing climate and produce hardier biofuel plants that could someday replace traditional fossil fuels.

ORGANISMS AS COMPUTERS

To tweak an organism, though, researchers need a basic understanding of how it works, and the molecular biology advances of the 1960s through the 1990s laid that foundation. The ability to cut DNA with restriction enzymes and paste, or ligate, it into new locations to then make multiple copies using the polymerase chain reaction, helped scientists determine what various pieces of genetic material do inside a cell. In contrast, traditional genetic breeding strategies offer much less control, are lower-throughput, and take much longer to obtain a result.

Most genes are not on constantly—instead, their expression is carefully regulated in time and space. Promoters, stretches of DNA that come before a gene, contain sequences that allow different regulatory proteins to bind. For example, repressors reduce or halt gene expression, whereas activators and enhancers boost expression. The combination of thousands of genes turning on or off, expressing at different levels at certain times and locations gives a cell, a tissue, or an entire organism its identity.

As research teams learned more about genes, the findings started to paint a picture that looked more and more like a computer, which got the attention of engineers. "Synthetic biology had a lot of electrical engineers at the start of the discipline," says Brophy. "They saw this correlation between how biological systems make decisions and how people program."

Computers provided a wealth of inspiration. They are made up of billions of logic gates within circuits that perform Boolean functions—just like setting up an internet search—to translate 1s and 0s into an action, or an output. Similarly, synthetic biologists have developed logic gates for many genes in many cells, from microbes to human cells.

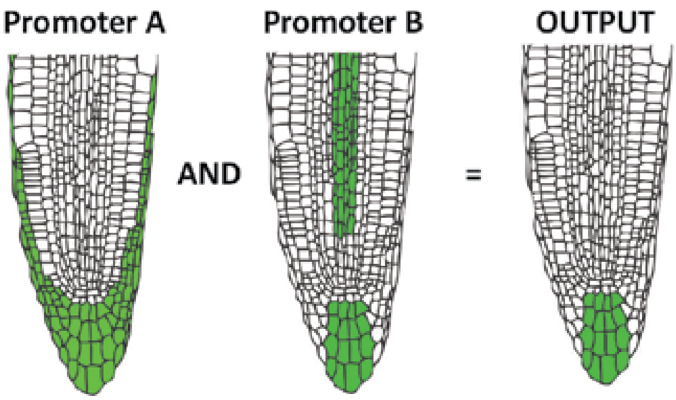
One of the most common logic gates is the "AND" gate, which combines inputs from multiple genes. For example, if there are two inputs and they are both active, then an action occurs. If only one or none of the inputs is active, then there is no action. In gene-speak, if two genes in an "AND" logic gate are turned on by activators, then the genes are both expressed. But, if only one of the genes is activated, then there is no expression. Combining logic gates gives researchers spatial and temporal control over gene expression.

FROM MICROBES TO PLANTS

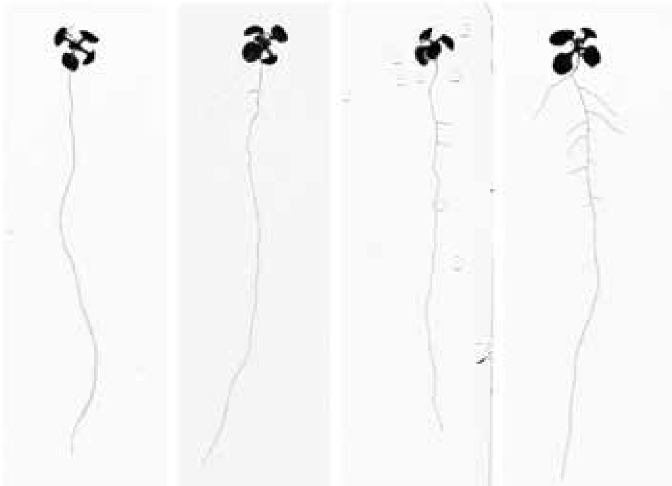
Synthetic biology was originally performed with microbes, and that is how Brophy got her start. "Bacteria has enabled some incredible synthetic biology," says Brophy. "We have been able to really precisely control gene expression to build up some pretty complex synthetic genetic programs."

Brophy developed technology to engineer soil bacteria to help plants but was unable to implement it in the field because of regulatory restrictions. "So, I thought, what if we can do the same type of engineering, but in the plants, instead of trying to engineer microbes to support plant health?" she explains.

Genetically modified plants are not without their controversies either, but as Brophy notes, the regulatory pathway for using them is under consideration. Some countries, including the US, allow commercial production of plants made with limited genetic changes and with genetic material from other organisms, though plants made with synthetic biology approaches are not on the market yet (http://tinyurl.com/59ba6aar). But people living in other areas like the UK are much more cautious about such manipulations, especially when it comes to the introduction of non-plant DNA (http://tinyurl.com/52tu93vw).



An example of gene expression (green) in roots with an "AND" logic gate ("OUTPUT"). Source: Jennifer Brophy



***A. thaliana* plants with logic gates that exhibit modified lateral root growth.** Source: Jennifer Brophy

By thinking of plants as computers, Brophy engineered a set of logic gates to perform specific functions. "We wanted to be able to take known tissue-specific promoters and combine their activity using logic gates to generate new patterns of expression across tissues in the plant, which would allow you to very precisely choose where to express genes," she says.

To minimize interference from the natural transcription system, the gates included some synthetic sequences at the promoter that were activated by synthetic activators or repressed by synthetic repressors. She transiently expressed these parts in the leaves of *Nicotiana benthamiana*, a relative of the tobacco plant—a rapid system that gave results in just a couple of days.

Once these tools were constructed and tested in *N. benthamiana* leaves, they were expressed in the model plant *Arabidopsis thaliana* for a more detailed characterization in a full plant. Of the eight gates tested, half worked well but half did not. Brophy redesigned the ones that did not work and was eventually able to improve all eight. In a real test of the approach, she used them to modify lateral root growth off the primary root, which grows straight down into the soil. Some gates resulted in barely any root growth on the sides, whereas other gates resulted in much more lateral growth, demonstrating the degree of control researchers could have over plant structures with this technology.

This was one of the first times that such complex programming had been achieved in a multicellular organism. "I was really excited about developing this suite of tools for circuit construction and using it to control spatial patterns," says Brophy. "It was a challenge to show that these circuits can function across so many different cells."

TOWARD A MORE SUSTAINABLE FUTURE

Realizing how useful Brophy's tools could be, a team of researchers funded by a long-running Department of Energy grant approached her to see if she would collaborate with

Sorghum bicolor. Source: iStockphoto.com

them on a biofuel project. The team wanted to increase water-use efficiency in grasses that could be used to produce biofuels.

One of the main drivers of climate change is the burning of fossil fuels, which releases greenhouse gases into the atmosphere, where they trap heat and warm the air. Although biofuels made from plants, algae or even animal fats could provide a renewable energy solution that would release little or no greenhouse gases, they require land to grow on, and sometimes are grown on land that could be used for food crops (http://tinyurl.com/4rdtfcsv). Improving how biofuel grasses use water could allow them to grow on lower-quality soil, reducing competition with food crops.

And all crops will be affected by climate change. The rate of warming has increased by a factor of three since 1982, and the last decade was the warmest 10 years on record, according to the National Oceanic and Atmospheric Administration (http://tinyurl.com/4kzxzuj2). With climate change comes more extreme weather events, such as droughts and floods, that occur more frequently and last longer than in the past (http://tinyurl.com/yt88k6fv). Agricultural crops can adapt to some degree, but most cannot change their root or leaf structures rapidly enough to keep pace with changing conditions.

Tamborski took on the challenge of implementing and optimizing Brophy's logic gates in biofuel grasses, and she started with testing them in a protoplast system. Protoplasts are plant cells that have had their protective cell walls removed so that genetic material can more easily pass into them. It is a rapid way to analyze the effects of various experimental conditions, so instead of waiting weeks for whole plants to grow, Tamborski transferred the logic gates into grass protoplasts in high-throughput, 96-well plates and obtained large amounts of data in about 24 hours.

But *A. thaliana* and biofuel grasses are very different types of plants that diverged from each other millions of years ago. Because of this, Tamborski had to modify the promoter and other sequences so they could work better in grasses.

With protoplasts, Tamborski tested "BUFFER" gates, as well as a "NOT IMPLIES" gate, which subtracts the overlapping area of expression. She found that, as in *A. thaliana*, expression levels could be tuned by making changes to the activators and to the promoter sequences that they bind. "Very nicely, we were able to see that we are indeed able to reconstruct most of the things we saw in *Arabidopsis thaliana* in the protoplasts and get very nice control of gene expression strength," she says.

So far, the experiments have only been performed with protoplasts of a model biofuel grass called *Setaria viridis*, but Tamborski has started to run the same battery of tests with protoplasts of a grass used in the field called *Sorghum bicolor*. She will also be testing the gates in sorghum plants and, in another collaboration, they will be venturing into wheat. In addition, they will dig out from the roots and implement logic gates in parts of the plant that grow above ground.

Positive results in wheat would show that logic gates could help agriculturally relevant plants survive changing climate conditions, which could also help humans. "If we can make all of the crops we grow more water-use efficient, then we could have an even more sizable impact on agricultural sustainability," says Brophy.

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