

PLANT SCIENCE

Plant synthetic biology takes root

Applying the basic principles of synthetic biology to plants shows progress

By June I. Medford¹ and Ashok Prasad²

The evolution of computers and electronics from bulky room-size devices to the sleek devices of today has relied on a well-honed strategy: Use parts whose behaviors are quantitatively defined and assemble complex systems from simpler modules. Synthetic biology aims to bring this approach to living organisms (1, 2), and it is already starting to happen in plants. A sensor and response system can be designed, sensor proteins programmed in a computer, encoded in a plant, and empowered with such properties as memory and amplification (3). Using genetic information and mathematical analysis allows one to design predictable and quantitative functions in plants, comparable to those of integrated electronic circuits. Just as integrated circuits can be reused in diverse electronic devices, from cell phones to aircraft, synthetic biology components should function predictably in different synthetic circuits and plants.

What sets synthetic biology apart from “engineering” plants through transgenic technology is that the former aims for predictable biological functions through the use of mathematical analyses, modeling, and orthogonal parts. It therefore relies on quantitatively characterized gene components such as transcriptional promoters, terminators, and proteins (e.g., translational modifiers). Like electronics, synthetic biology assembles quantitatively defined parts in particular ways (with standard rules for their connections) to produce biological functions such as memory, amplification, and precise switches.

Synthetic gene circuits are designed to act independently of the organism’s natural systems. They can be designed in silico and then assembled to yield the correct behavior (4). For example, gene circuits that use Boolean logic (values are reduced to “true” or “false”) have been produced in bacteria by modeling behavior in silico and producing simple logic gate functions to control biological signals and responses. Thus, in *Escherichia coli*, many synthetic circuits starting from a genetic toggle switch (5) to genetic programs using layered logic gates (6) have been engineered by this procedure.

¹Department of Biology and ²Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO 80523, USA. E-mail: june.medford@colostate.edu

If genetic toggle switches and genetic logic gates could be produced in plants, precise control of plant traits would be feasible. A bioenergy crop such as sorghum could devote its resources to biomass production prior to activating a synthetic toggle switch that diverts the plant’s resources to oil accumulation. Basic plant studies also could benefit from a synthetic toggle switch to help elucidate complex gene functions. Such logic circuits in plants have not yet been achieved.



Sentinel plant. A sensor protein was computationally designed and linked to downstream gene components to enable a plant (*Nicotiana tabacum* shown) to detect an external ligand (100 nM TNT) and respond with a visual display (such as loss of chlorophyll) (3).

The vast majority of synthetic genetic circuits have been produced in bacteria, and designing similar circuits for plant platforms faces the greater complexity of plant genomes and protein networks. Moreover, there are currently only a handful of quantitatively characterized plant components with different rules for assembling them into functional expression units in monocots and dicots. In addition, unlike bacterial plasmids, plant components may be affected by the contextual information provided by the chromosome position.

Plant biologists have long understood positional effects from variable transfer DNA (T-DNA) insertion in the chromatin. The tumor-inducing plasmid of bacteria such as *Agrobacterium tumefaciens* has been used in plant genetic engineering in which tumor-promoting genes are removed and a gene of interest inserted for stable integration into plants (7). Less well appreciated is the need for chromatin insulators within a synthetic gene circuit to rigorously isolate one gene-encoded function from another. These positional effects are just beginning to be understood through approaches such as the use of unique chromosomal posi-

tions or “landing pads” to use when quantitatively characterizing plant components. Multiple technologies for targeted chromosomal integrations such as zinc fingers, TALENs, and CRISPRs exist for this type of approach in plants (8, 9).

A further challenge in addressing the context-dependent behavior of synthetic circuits in plants is the unavoidable interaction of synthetic proteins and genes with the host system. For example, host DNA can act as a load on a synthetic gene cir-

cuit and show altered behavior (e.g., slower switching in a toggle switch) (10). Similarly, orthogonal synthetic circuits may be coupled to the host system through their dependence on the host system for synthesis and degradation. Hence, if a synthetic circuit functions correctly in a model plant like *Arabidopsis thaliana*, the effects of the context should be considered when transferring that circuit to a species such as soybean or corn.

One approach to manage the challenges of using plants as a synthetic biology platform is to refactor the genetic process of interest, that is, to simplify design parameters and remove inefficiencies while still accomplishing the desired function. For example, the nitrogen fixation pathway from *Klebsiella oxytoca* was refactored by removing noncoding DNA and nonessential genes, connecting the remaining genes to orthogonal promoters, and altering the DNA sequences to be as different from the original as possible while still coding for the same proteins (11). The synthetic nitrogen fixation cluster could then be introduced into a plant’s chloroplast or mitochondria.

Adjusting to the fertility bust

What is the best response to declining populations?

By Timothy M. Smeeding

Whereas electrical circuits are physically connected with wires, gene functions often require diffusible factors (e.g., proteins). Hence, producing a predictable function is more challenging in biological systems and subcellular compartmentalization must also be considered. Simplifying complex signaling systems in plants allowed the production of the first synthetic signal transduction system (12), linking the presence of external substances [such as trinitrotoluene (TNT)] to internal responses with only two or three genes; when coupled with computational protein redesign, the result was “computerized” detection technology in plants, which may have applications as environmental sensors (3, 13) (see the figure).

Another major challenge is that quantitative characterization of genetic components for a multicellular plant in isolated plant cells (such as mesophyll protoplasts) has limitations, particularly because cultured plant cells do not have the cell-cell interactions found in differentiated tissues. Nonetheless, progress has been made in transient expression methods that enable the efficient expression of multiple genes within the same plant cell over a time scale of days (14, 15). This has led to the production of macromolecular complexes, such as virus-like particles that have potential use in nanotechnology and as vaccines. However, it is important that genetic controls be introduced into whole plants to assess their behaviors under “real life” conditions.

By applying theory-driven engineering approaches developed for electronics to plant biology, we can not only uncover the natural genetic circuits behind complex gene regulations in plants, but we could also design traits that are new to evolution and beneficial to humanity. The future is looking green. ■

REFERENCES

1. D. Endy, *Nature* **438**, 449 (2005).
2. E. Andrianantoandro *et al.*, *Mol. Syst. Biol.* **2**, 2006.0028 (2006).
3. M. S. Antunes *et al.*, *PLOS ONE* **6**, e16292 (2011).
4. J. A. Brophy, C. A. Voigt, *Nat. Methods* **11**, 508 (2014).
5. T. S. Gardner, C. R. Cantor, J. J. Collins, *Nature* **403**, 339 (2000).
6. T. S. Moon, C. Lou, A. Tamsir, B. C. Stanton, C. A. Voigt, *Nature* **491**, 249 (2012).
7. C. N. Stewart *et al.*, Eds., *Plant Transformation Technologies* (Blackwell, West Sussex, UK, 2011).
8. V. K. Shukla *et al.*, *Nature* **459**, 437 (2009).
9. Z. Feng *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 4632 (2014).
10. S. M. Lyons, W. Xu, J. Medford, A. Prasad, *PLOS Comput. Biol.* **10**, e1003533 (2014).
11. K. Temme, D. Zhao, C. A. Voigt, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 7085 (2012).
12. M. S. Antunes *et al.*, *Mol. Syst. Biol.* **5**, 270 (2009).
13. K. J. Morey *et al.*, *Methods Enzymol.* **497**, 581 (2011).
14. S. D. Yoo, Y. H. Cho, J. Sheen, *Nat. Protoc.* **2**, 1565 (2007).
15. F. Sainsbury, G. P. Lomonosoff, *Curr. Opin. Plant Biol.* **19**, 1 (2014).

10.1126/science.1261140

Fertility in the United States is at an all-time low, having reached a rate of 1.86 children per woman of child-bearing age in 2013 (1). Researchers warn of the worldwide “low-fertility trap” (2), and the population of Europe is projected to decline in the next 25 years (3). Should we be worried? On page 229 of this issue, Lee *et al.* (4) argue that moderately low fertility and modest population decline favor a higher living standard. They are not alone in urging restraint to concerns about population aging (5) but have painstakingly assembled a new data set to make their point very clearly. Meanwhile, Gerland *et al.* contend on page 234 of this issue (6) that the world population will still grow substantially and is unlikely to stop growing in this century. However, almost all of the projected population increase by 2100 is confined to Sub-Saharan Africa, with almost all other continents and major countries experiencing declining rates of population growth and rising ratios of elders to workers.

There was once a “demographic dividend” from high fertility that has now largely disappeared. Substantial reductions in child and infant mortality rates meant that women and families needed to have fewer children to reach their desired number of offspring. Falling child mortality led to a higher life expectancy and, at least initially, a larger working-age population. More workers led to higher fiscal balances in intergenerational transfer systems, such as most social retirement systems in rich countries. For a time there were more workers than elders, raising revenue per elder and benefitting one generation (the older one) at the expense of another.

The policy problem of an aging population is surely one to be recognized. The U.S. “baby boom” between 1946 and 1960 created a huge demographic dividend for the parents of these children, not to mention a larger number of offspring to care for these persons in old age. But then fertility declined rapidly, and the number of elders (now the early baby boomers) became large relative to the number of workers as lower fertility reduced the growth rate of the labor force. At the same time, continuing improvements in old-age mortality led to faster growth of the elderly population. This U.S. baby bust has led to projections of substantial long-term

changes in federal spending priorities and shortages in trust funds for Social Security and Medicare (7).

In addition to this common policy problem, what else matters in aging populations (see the figure)? Well, in a word, behavior. First, savings, work, and both physical and human capital formation may change. Older generations, with an extended period of retirement and fewer workers to support them, can and often do save more and work lon-

“... as governments confront population aging, they must also address a number of related policy issues: immigration, inequality, education, and gender/family equity in the workplace ...”

ger. That saving will lead to faster economic growth if it increases physical productive capital. This growth dividend can produce a larger economic pie for all, especially if greater education investments are made to increase human capital in the younger and smaller generation to complement the technological change that comes with physical capital growth. But this is not always the case; in fact, the United States may be losing the race between investments in human capital (education) and physical capital (technology) (8). And so we have a different worry: underinvestment in education.

Second, private transfers from the old to the young can offset many of these intergenerational imbalances. The generation who feels their living standards are highest—that is, current elders—may transfer more money and educational opportunity to those who are younger, as has been the case in many rich nations in recent decades. These transfers do, however, depend on the distribution of wealth among the older populations compared to younger ones. Growing wealth inequality (9) means that wealthy households

Department of Public Affairs and Economics, University of Wisconsin, Madison, WI 53706, USA. E-mail: smeeding@lafollette.wisc.edu