FEATURES

You Reap What You Edit: Synthetic Gene Circuits in Plants

Courtesy of Adobe Stock.

Sohum Sukhatankar ’28

Introduction

A sunflower turns to track the sun, a Venus flytrap snaps shut at a slight touch, and a desert plant responds to minute changes in humidity. Though plants cannot “see” or “hear” in the conventional sense, they nonetheless possess the ability to detect environmental stimuli including humidity, acidity, heat, and more (**Fig. 1**). Advances in molecular biology are now enabling scientists to harness plants' environmental responses by integrating them with systems of synthetic gene circuits, which continuously update gene expression via logic gate-like structures.

Historically, genetic plant modification has relied on transgenes—genes from other species inserted into a plant’s genome to enhance traits like nutritional value or pathogen resistance. For instance, golden rice has genes from the soil bacterium *Agrobacterium tumefaciens* that improve its nutritional content (Tang et al., 2009). However, transgenic plant creation is not without its flaws. There is no guarantee that a transgene will express consistently at the desired level or in the right cellular location, as transgenic plants express these transgenes continuously in all cells. Synthetic gene circuits offer a solution by enabling plants to dynamically modulate gene expression in response to specific environmental conditions through a network of logic gates. Unlike transgenes, synthetic gene circuits can function by causing epigenetic modifications rather than altering DNA itself, minimizing the risk of gene transfer to wild plants and reducing the chance of unintended effects.

Plants as Genetic Sensors, Processors, and Actuators

Clarifying plant genetics is critical for understanding the construction of environment-sensitive genetic synthetic circuits. Unlike bacteria—in which prototypical gene circuits were developed—plants lack operons, or groups of linked genes that can modulate the activity of other genes based on various input signals. Instead, they have a variety of signaling components (e.g., protein cascades, which serve to amplify signals) that connect across pathways to create a complex network of related information (Andres et al., 2019). The inner workings of plants can be harnessed by coupling these mechanisms to three synthetic gene circuit components: sensors, processors, and actuators, with the bulk of the customizability arising from the processor design.

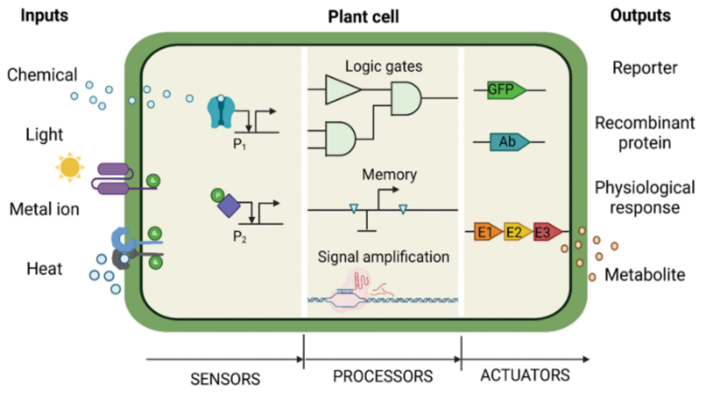

Figure 1. An overview of the structure of synthetic gene circuits, wherein a variety of inputs pass through a combination of plant and human-engineered components to produce a desired output (Vazquez-Vilar et al., 2023).

Sensors

Synthetic gene circuits can better measure external stimuli via sensor modules, which record environmental signals like light level, chemical concentration, or humidity, converting these signals into transcriptional outputs. A well-studied example is the auxin sensor, which couples green fluorescent protein (GFP) activity to the amount of auxin, a plant hormone produced in response to changes in light or soil pH (Vazquez-Vilar et al., 2023). By combining sensors for different plant hormones and enzymes to produce distinct outputs—such as fluorescence—researchers can assess the effects of a plant's environment on its behavior.

Processors

While sensors detect external signals, processors transform these signals into transcriptional outputs, amplifying sensor signals and linking them to normally unrelated genes. Processors achieve this by coupling two protein domains to form synthetic transcription factors (TFs), which are proteins that regulate gene expression. One domain regulates specific genes, while the other binds to particular DNA sequences. This "chimerizing" of TFs—combining sequences from different plants or species—resembles traditional transgenic plant processes but influences gene expression rather than editing the content of genes. CRISPR technology has further simplified this synthesis by enabling the DNA-binding domain of TFs to pair with the dead Cas9 (dCAS9) protein (Piatek et al., 2015). Unlike Cas9, which cleaves DNA, dCas9—directed by guide RNA—only binds to a target gene's promoter sequences to decrease further transcription.

A recent advance of CRISPR interference in synthetic gene circuits involves two binding sites (A and B) for dCas9 in the luciferase gene's promoter sequence (Khan et al., 2022). Each site is targeted by a guide RNA triggered by a distinct sensor input, independently recruiting dCas9 to one of the sides only. The binding of dCas9 to either site represses the promoter, meaning that the promoter is active *if and only if* neither guide RNA is expressed. This system can be modeled by the logical operator NOR, where an output of TRUE means that the promoter has been activated by guide RNA information. To illustrate, imagine charging a computer: it will charge (NOR is true) *only if* both the charger is plugged into the computer's port (A is false) and the wall outlet (B is false). Conversely, if either connection is unplugged (A or B is true), the computer won't charge (NOR is false). NOR(A, B) is true *if and only if* both A and B are false **(Fig. 2)**. As it turns out, the NOR operator is "functionally complete," meaning that all logic gates can be expressed as a combination of NOR gates. If this approach can be extended to other genes or gene combinations with suitably chimerized activators and repressors, researchers could theoretically engineer optimal gene expression for plants based on true/false responses to an unlimited array of environmental factors.

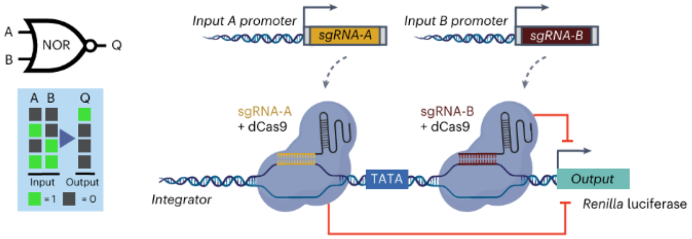

Figure 2. The circuit synthesized by Khan et al. (2022). The expression of the Renilla luciferase gene (the output) is only activated when neither promoter (A, B) is bound. Only when both outputs are False (A = 0, B = 0) does the output become true (Q = 1).

Actuators

Processor outputs are directed to a series of actuators—genes responsible for regulating various plant traits including metabolic enzymes, ion channels, and defense proteins. Actuators can also take the form of fluorescent proteins. An experiment by Vazquez-Vilar et al. (2017) quantified the level of transcription via the relative amount of the fluorescent protein luciferase in different regions of the plant at different times. Measuring this activity allowed the circuit to be fine-tuned to achieve the desired reporter protein expression levels.

Flaws in Current Methods and Timeline for Implementation

Significant challenges remain before synthetic gene circuits can be implemented in plants on a large scale. Since this method requires a series of logic gates—each of which should return either TRUE or FALSE—to activate specific cells, intermediate levels of expression due to imperfect orthogonality can make the truth value of a gate ambiguous, potentially producing the opposite of the desired value for all subsequent gates.

Additionally, adapting these circuits across different species requires specific tailored modifications. Most research has focused on thale-cress (*Arabidopsis thaliana*), a preferred model organism due to its small genome size and short generation time (Vazquez-Vilar et al., 2023). Transient expression via the bacteria *Agrobacterium*, which works extremely well in transforming *A. thaliana*, has been the method of choice for observing the effects of gene circuits, but stable crops other than A. thaliana will likely need alternative hosts and methods to retain the circuits over the long term.

Other Features and Applications of Synthetic Gene Circuits

In synthetic gene circuits, maximizing specificity and orthogonality—minimizing cross-interaction between synthetic and endogenous plant genes—is crucial. This design prevents interference, both between natural responses to stimuli and circuits as well as between different circuits. High orthogonality allows for more predictable responses and reduces the risk of harmful cross-talk within host cells. One strategy for orthogonality is to incorporate components from non-plant sources such as the bacteria-derived dCas9 or the firefly-derived luciferase. Another strategy is the randomization of the DNA elements within a processor (Belcher et al., 2020). While past studies combined DNA elements within the same organism for the processor design, researchers found that the use of DNA elements from both yeast and plants had the potential to produce both a wider range of transcriptional strength and a more orthogonal circuit.

Some researchers are working to adapt synthetic gene circuit technology for use in humans. The mammalian immune system is generally more accessible than that of plants, opening possibilities for applications like chimeric antigen receptor T cell therapy (CAR-T therapy), where chimerized T cells express antigen-targeting receptors on their surfaces (Li et al., 2023). These receptors can then help trigger anti-cancer responses in affected areas of the body. Much like in plants, mammalian circuits prioritize orthogonality to avoid interference with the body's natural systems. However, early trials of CAR-T therapy have occasionally led to adverse effects and even death. In one case, inadequate orthogonality of chimeric T cells led to a "cytokine storm," as the patient's immune system mistakenly attacked the patient (Morgan et al. 2010). For synthetic gene circuits to be safely and broadly implemented, researchers must ensure near-total predictability in circuit behavior, especially given the complexity of potential interactions with thousands of endogenous human genes.

Conclusion

The potential impact of synthetic gene circuits in agriculture is immense. If integrated across various crops at a large scale, these circuits could increase plant resilience towards pathogens and severe weather events by upregulating genes that control immune responses and other defenses precisely when needed. At the same time, scientists have the agency to make sure any of these changes do not affect the plant's environment if something goes awry - they can simply inactivate the circuit by reprogramming the involved guide RNAs (Khan et al, 2022).. By combining existing genes within the plant with a highly customizable system of circuits, researchers are optimizing the world around us via computer logic in a sustainable and eco-friendly way. As gene circuit technology develops and is integrated into more complex organisms, these systems may be harnessed to make many aspects of human life easier, starting with crop engineering.

References

Andres, J., Blomeier, T., & Zurbriggen, M. D. (2019). Synthetic Switches and Regulatory Circuits in Plants. Plant physiology, 179(3), 862–884. https://doi.org/10.1104/pp.18.01362

Belcher, M.S., Vuu, K.M., Zhou, A. et al. (2020). Design of orthogonal regulatory systems for modulating gene expression in plants. Nat Chem Biol 16, 857–865 (2020). https://doi.org/10.1038/s41589-020-0547-4

Khan, M. A., Herring, G., Zhu, J. Y., Oliva, M., Fourie, E., Johnston, B., Zhang, Z., Potter, J., Pineda, L., Pflueger, J., Swain, T., Pflueger, C., Lloyd, J. P. B., Secco, D., Small, I., Kidd, B. N., & Lister, R. (2024). "CRISPRi-based circuits to control gene expression in plants". Nature biotechnology, Advance online publication. https://doi.org/10.1038/s41587-024-02236-w

Li, H. S., Israni, D. V., Gagnon, K. A., Gan, K. A., Raymond, M. H., Sander, J. D., Roybal, K. T., Joung, J. K., Wong, W. W., & Khalil, A. S. (2022). Multidimensional control of therapeutic human cell function with synthetic gene circuits. Science (New York, N.Y.), 378(6625), 1227–1234. https://doi.org/10.1126/science.ade0156

Morgan, R. A., Yang, J. C., Kitano, M., Dudley, M. E., Laurencot, C. M., & Rosenberg, S. A. (2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Molecular therapy : the journal of the American Society of Gene Therapy, 18(4), 843–851. https://doi.org/10.1038/mt.2010.24

Piatek, A., Ali, Z., Baazim, H., Li, L., Abulfaraj, A., Al-Shareef, S., Aouida, M., & Mahfouz, M. M. (2015). RNA-guided transcriptional regulation in planta via synthetic dCas9-based transcription factors. Plant biotechnology journal, 13(4), 578–589. https://doi.org/10.1111/pbi.12284

Tang, G., Qin, J., Dolnikowski, G. G., Russell, R. M., & Grusak, M. A. (2009). Golden Rice is an effective source of vitamin A. The American journal of clinical nutrition, 89(6), 1776–1783. https://doi.org/10.3945/ajcn.2008.27119

Vazquez-Vilar, M., Quijano-Rubio, A., Fernandez-Del-Carmen, A., Sarrion-Perdigones, A., Ochoa-Fernandez, R., Ziarsolo, P., Blanca, J., Granell, A., & Orzaez, D. (2017). GB3.0: a platform for plant bio-design that connects functional DNA elements with associated biological data. Nucleic acids research, 45(4), 2196–2209. https://doi.org/10.1093/nar/gkw1326

Vazquez-Vilar, M., Selma, S., Orzaez, D., The design of synthetic gene circuits in plants: new components, old challenges, Journal of Experimental Botany, Volume 74, Issue 13, 18 July 2023, Pages 3791–3805, https://doi.org/10.1093/jxb/erad167