Focus Issue Editorial: Synthetic Biology

Synthetic biology (SynBio) is a conceptual and operational revolution (Church et al., 2014) that's coming soon to a branch of plant science near you, if it's not there already (Liu and Stewart, 2015). The Synthetic Biology Focus Issue sets out to spread this disruptive news.

SynBio is a transformative combination of DNA technology, engineering principles, and computational tools that makes it possible to design new life processes and to repurpose existing natural ones for useful purposes (Purnick and Weiss, 2009). SynBio will profoundly impact and empower how plant science is done and how plant science is used to sustainably solve global problems. SynBio is already creating new jobs and is likely to keep doing this for decades (Delebecque and Philp, 2015).

SynBio is powerful for conceptual and operational reasons. The enormous conceptual power of SynBio is to open access to the vast "design space" that plants, and nature in general, have not explored (Bhatia et al., 2017). By discovering and deploying useful design space that evolution has "missed," SynBio enables plants: to make familiar compounds by new pathways, and make new-to-nature compounds; to supply genes needed to make high-value compounds in microorganisms; to manipulate familiar morphological structures, and build totally new-to-nature ones; to respond in new ways to old stimuli, and sense and respond to totally new stimuli; and to be managed based on weather forecasts and other predictions that plants cannot make themselves.

The transformative operational power of SynBio lies in its drive to industrialize biology: that is, to replace high-skill, slow, and costly artisanal work such as cloning and custom assays with computationally guided, automated, and standardized engineering procedures (Cameron et al., 2014; Chao et al., 2017). Although the industrial-scale engineering potential of SynBio is far from fully realized at this point (Davies, 2019), it is very much here to stay and has begun to dictate major changes in biology education and training (National Research Council, 2015). Whether you are a trainee or a trainer, these trends are seriously worth considering for your future career's sake.

A hallmark of SynBio is to rethink biological molecules, genes and proteins, as engineering "parts" that can be standardized, quantitatively characterized, and used to build a range of devices, much as standard resistors and capacitors are used as components in countless different electrical circuits (de Lorenzo and Schmidt, 2018). In the case of metabolic pathways, for instance, enzyme parts from plants or other organisms can be used as is and combined in novel ways to build new pathways, or used as the starting point for directed evolution to create an enzyme part with a new-to-nature activity (Erb et al., 2017). Directed evolution is one of SynBio's most iconic achievements, as recognized by the 2018 Nobel Prize in Chemistry to Frances H. Arnold "for the directed evolution of enzymes" (Ranganathan, 2018). However, reconceiving genes and proteins as potential SynBio parts spotlights how massively incomplete our "parts lists" for organisms still are. Even in the minimal *Mycoplasma mycoides* JCVI-syn3.0 genome, 149 genes out of a total of 473 (32%) have no known biological function (Hutchison et al., 2016), and the situation is far worse in plants, with Arabidopsis (*Arabidopsis thaliana*) having ~7,000 enzymes and transporters of unknown function (Niehaus et al., 2015). The curiosity-driven research that has been accustomed to sifting through these proteins to assign function with few if any concrete objectives can now be reframed and refocused as purposeful bioprospecting or "parts discovery" for SynBio, a highly defensible justification for continued public support of what can seem to some like an aimless academic exercise. Principal investigators might want to consider this argument.

The collection of Update Reviews, Research Articles, and Breakthrough Technologies Articles in this Focus Issue cover the above conceptual and operational possibilities and more, as we now detail.

Starting with SynBio-enabled engineering of plant metabolism, three Update Reviews and one Research Article cover photosynthesis and plastid metabolism. The Update by Leister (2019) explores progress and prospects in applying SynBio to the light reactions of photosynthesis, both to enhance the efficiency of light use in crops and to couple the light reactions in novel ways to enzymes or nonbiological components that use the reducing power from the light reactions (see also the News and Views article in this issue by Moses, 2019). The Update by Boehm and Bock (2019) explains the promise of the plastid genome as a platform for engineering plant metabolism, describes tools and technologies for plastid SynBio, and highlights challenges for future research in this area. The Research Article by Occhialini et al. (2019) expands on tools and technologies by describing the development and validation of a modular chloroplast cloning system, MoChlo, for constructing heterologous metabolic pathways in plastids. The Update by Weber and Bar-Even (2019) discusses the huge potential for SynBio to raise crop yields by increasing the CO_2 concentration around Rubisco to reduce photorespiration and by rerouting photorespiratory metabolism via synthetic bypasses or new pathways that convert photorespiration from a carbon-loss process into a carbon-gain process.

Two Update Reviews and a Research Article then look toward the SynBio-enabled future of plant metabolic

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engineering. Mitchell and Weng (2019) review the structure-function relationships of cytochrome P450 monooxygenases and other plant oxygenases and show how these enzymes are a versatile toolset for SynBio to develop biocatalysts and manipulate plant traits. The review by Wurtzel (2019) explores how applying SynBio to carotenoid pathways could alter plant form and function for agricultural and industrial purposes and emphasizes the ongoing need for discovery research to drive the SynBio era forward. Illustrating the utility of discovery research, Sun et al. (2019) report success in a targeted parts-prospecting project to discover genes from which to start the directed evolution of a highly efficient thiamin synthesis enzyme.

Two articles come from the very active and commercially important field of rebuilding plant biosynthetic pathways in tractable laboratory microbes. The Update by Pyne et al. (2019) provides a broad overview of the field and its challenges, with particular emphasis on pathways for total de novo synthesis of high-value plant isoprenoids, alkaloids, phenylpropanoids, and polyketides from sugar feedstocks. The Research Article by Kallscheuer et al. (2019) first establishes that salidroside (a phenylpropanoid glucoside from raspberry [*Rubus idaeus*]) is bioactive, then details the reconstruction of salidroside biosynthesis in *Saccharomyces cerevisiae* and *Corynebacterium glutamicum*.

Two Updates and three Articles cover orthogonal (i.e. nonnative) synthetic sensing and regulatory circuits and their application to plant development. The review by Andres et al. (2019) introduces and illustrates the key concepts of synthetic switches, logic gates, and regulatory circuits, which leads neatly to the Research Articles by Iacopino et al. (2019), on the design and testing of a synthetic oxygen sensor for plants based on bioengineered versions of a mammalian oxygen sensor, and by Schreiber et al. (2019), on design and optimization of a two-component AND-gate system for synthetic circuits in plants based on bacterial transcription activator-like effectors. Wright and Nemhauser (2019) review how SynBio can help uncover the parts and logic mediating plant development, leading to predictive models that specify the molecular circuitry needed to change cell state. This review connects to the Breakthrough Technologies Article by Faden et al. (2019) on the use of a temperature-switchable version of a toxic bacterial protein to control trichome development as a test case.

Lastly, three Updates and two Articles address core technologies that enable SynBio. One such technology, and another hallmark of full-blown SynBio, is computational design, which Küken and Nikoloski (2019) review in relation to synthetic metabolic pathways using a case study approach. Two further core SynBio technologies are protein engineering and directed evolution, both of which are reviewed by Engqvist and Rabe (2019). Another core technology is the construction of synthetic enzyme complexes, comprehensively reviewed by Smirnoff (2019) and illustrated by Camagna et al. (2019) in a Research Article detailing a fused-enzyme metabolon to boost flux to phytoene synthesis.

In closing their Update, Weber and Bar-Even (2019) quote Nobel Peace Prize winner Norman Borlaug: "Then I wake up and become disillusioned to find that mutation genetics programs are still engaged mostly in such minutiae as putting beards on wheat plants and taking off the hairs." Weber and Bar-Even then add on their own account, "we should leave the minutiae behind and reap the full potential of synthetic biology to overcome one of the major challenges of the 21st century, sustainably feeding a growing population without destroying the environment." Amen.

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LITERATURE CITED

Andres J, Blomeier T, Zurbriggen MD (2019) Synthetic switches and regulatory circuits in plants. Plant Physiol **179:** 862–884

Bhatia SP, Smanski MJ, Voigt CA, Densmore DM (2017) Genetic design via combinatorial constraint specification. ACS Synth Biol **6:** 2130–2135

Boehm CR, Bock R (2019) Recent advances and current challenges in synthetic biology of the plastid genetic system and metabolism. Plant Physiol **179:** 794–802

Camagna M, Grundmann A, Bär C, Koschmieder J, Beyer P, Welsch R (2019) Enzyme fusion removes competition for geranylgeranyl diphosphate in carotenogenesis. Plant Physiol **179:** 1013–1027

Cameron DE, Bashor CJ, Collins JJ (2014) A brief history of synthetic biology. Nat Rev Microbiol **12:** 381–390

Chao R, Mishra S, Si T, Zhao H (2017) Engineering biological systems using automated biofoundries. Metab Eng **42:** 98–108

Church GM, Elowitz MB, Smolke CD, Voigt CA, Weiss R (2014) Realizing the potential of synthetic biology. Nat Rev Mol Cell Biol **15:** 289–294
Davies JA (2019) Real-world synthetic biology: Is it founded on an engineering approach, and should it be? Life (Basel) **9:** E6
Delebecque C, Philp J (2015) Training for synthetic biology jobs in the new bioeconomy. http://www.sciencemag.org/careers/2015/06/training-synthetic-biology-jobs-new-bioeconomy
de Lorenzo V, Schmidt M (2018) Biological standards for the knowledge-based bioeconomy: What is at stake. N Biotechnol **40:** 170–180
Engqvist MKM, Rabe KS (2019) Applications of protein engineering and directed evolution in plant research. Plant Physiol **179:** 907–917
Erb TJ, Jones PR, Bar-Even A (2017) Synthetic metabolism: Metabolic engineering meets enzyme design. Curr Opin Chem Biol **37:** 56–62
Faden F, Mielke S, Dissmeyer N (2019) Switching toxic protein function in life cells. Plant Physiol **179:**
Hutchison CA III, Chuang RY, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma L, et al (2016) Design and synthesis of a minimal bacterial genome. Science **351:** aad6253
Iacopino S, Jurinovich S, Cupellini L, Piccinini L, Cardarelli F, Perata P, Mennucci B, Giuntoli B, Licausi F (2019) A synthetic oxygen sensor for plants based on animal hypoxia signalling. Plant Physiol **179:** 986–1000
Kallscheuer N, Menezes R, Foito A, Silva M, Braga A, Dekker W, Sevillano DM, Rosado-Ramos R, Jardim C, Oliveira J, et al (2019) Identification and microbial production of the raspberry phenol salidroside that is active against Huntington's disease. Plant Physiol **179:** 969–985
Küken A, Nikoloski Z (2019) Design of synthetic metabolic pathways: Challenges and opportunities for plant synthetic biology. Plant Physiol **179:** 894–906
Leister D (2019) Genetic engineering, synthetic biology and the light reactions of photosynthesis. Plant Physiol **179:** 778–793
Liu W, Stewart CN Jr (2015) Plant synthetic biology. Trends Plant Sci **20:** 309–317
Mitchell AJ, Weng JK (2019) Unleashing the synthetic power of plant oxygenases: From mechanism to application. Plant Physiol **179:** 813–829
Moses T (2019) Shedding light on the power of light. Plant Physiol **179:** 775–777
National Research Council (2015) Industrialization of Biology: A Roadmap to Accelerate the Advanced Manufacturing of Chemicals. National Academies Press, Washington, DC
Niehaus TD, Thamm AM, de Crécy-Lagard V, Hanson AD (2015) Proteins of unknown biochemical function: A persistent problem and a roadmap to help overcome it. Plant Physiol **169:** 1436–1442
Occhialini A, Piatek A, Pfotenhauer AC, Frazier TP, Stewart CN Jr., Lenaghan SC (2019) MoChlo: A versatile modular cloning toolbox for chloroplast biotechnology. Plant Physiol **179:** 943–957
Purnick PE, Weiss R (2009) The second wave of synthetic biology: From modules to systems. Nat Rev Mol Cell Biol **10:** 410–422
Pyne ME, Narcross L, Martin VJJ (2019) Engineering plant secondary metabolism in microbial systems. Plant Physiol **179:** 844–861
Ranganathan R (2018) Putting evolution to work. Cell **175:** 1449–1451
Schreiber T, Prange A, Tissier AF (2019) Split-TALE: A TALE-based two-component system for synthetic biology applications in planta. Plant Physiol **179:** 1001–1012
Smirnoff N (2019) Engineering of metabolic pathways using synthetic enzyme complexes. Plant Physiol **179:** 918–928
Sun J, Sigler CL, Beaudoin GA, Joshi J, Patterson JA, Cho KH, Ralat MA, Gregory JF, Clark DG, Deng Z, et al (2019) Parts-prospecting for a high-efficiency thiamin thiazole biosynthesis pathway. Plant Physiol **179:** 958–968
Weber APM, Bar-Even A (2019) Improving the efficiency of photosynthetic carbon reactions. Plant Physiol **179:** 803–812
Wright RC, Nemhauser J (2019) How can synthetic biology help quantify and discover the unknowns in plant development? Plant Physiol **179:** 885–893
Wurtzel ET (2019) Changing form and function through carotenoids and synthetic biology. Plant Physiol **179:** 830–843