Review

CellPress

Special Issue: Manifesting Synthetic Biology

Enabling plant synthetic biology through genome engineering

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Synthetic biology seeks to create new biological systems, including user-designed plants and plant cells. These systems can be employed for a variety of purposes, ranging from producing compounds of industrial or therapeutic value, to reducing crop losses by altering cellular responses to pathogens or climate change. To realize the full potential of plant synthetic biology, techniques are required that provide control over the genetic code – enabling targeted modifications to DNA sequences within living plant cells. Such control is now within reach owing to recent advances in the use of sequence-specific nucleases to precisely engineer genomes. We discuss here the enormous potential provided by genome engineering for plant synthetic biology.

The importance of genome engineering for synthetic biology

Synthetic biology (see Glossary) is often hard to define because it encompasses a broad range of methodologies for manipulating and harnessing living systems. In simplest terms, synthetic biology combines science and engineering to design and construct new biological parts, devices, and systems [1]. One area of synthetic biology, and the focus of this review, is the generation of user-designed organisms. These organisms are created for a variety of purposes, ranging from producing valuable compounds that are ultimately purified away from the host to improving the response of an organism to the environment by designing genetic circuits that respond better to external cues. To fully practice in this area of synthetic biology one requires control over DNA sequences, from the *in silico* design and *in vitro* synthesis of standardized genetic elements to the *in vivo* manipulation of host DNA and gene expression.

There are now a wide variety of tools available for *in vivo* manipulation of the genetic material, including recombinases, integrases, RNAi technology, and sequence-specific nucleases, the latter being the focus of this review. Extraordinary advances in sequence-specific nuclease technology within the past 5 years have made it possible for most labs, even those with minimal molecular biology expertise, to precisely manipulate plant genomes, including altering DNA sequences and changing patterns of gene expression. We focus here on sequence-specific nucleases and how they have been used to create genetic modifications for synthetic biology projects. We also discuss future roles for these tools in plant synthetic biology, using examples from several projects, including the ongoing C_4 rice project, where photosynthesis in rice is to be completely redesigned for higher efficiency [2], and the nitrogen-fixing cereals project, where cereals are to be modified to uptake atmospheric nitrogen [3] (Box 1).

Why practice synthetic biology in plants?

Plants have largely been unexploited for synthetic biology, but they offer great potential. Plants are the most important source of the primary metabolites that feed the world (i.e., proteins, fatty acids, and carbohydrates) and they also produce a diverse array of secondary metabolites of value for medicine and industry. Further, there is a good understanding of plant systems biology, they are sessile, they can

Glossary

Gene targeting: a process that uses the homologous recombination pathway to introduce DNA sequence changes within genomes. Instead of using homologous sequence present on the sister chromatid or homologous chromosome, it is possible to 'trick' the cell into using a user-supplied donor molecule for repair [8]. Differences in sequences within the donor molecule, compared to the chromosomal target, will be copied and stably incorporated into the host genome.

Guide RNA (gRNA): Cas9 is targeted to a specific DNA sequence using a gRNA. This gRNA consists of two RNA molecules – a CRISPR RNA (crRNA) and a transactivating CRISPR RNA (tracrRNA). To reduce the complexity of the system for genome engineering, the crRNA and tracrRNA can be fused into a single gRNA.

Homologous recombination (HR): a DNA double-strand break (DSB) repair pathway. Repair is template-directed and uses homologous sequences present on the sister chromatid, homologous chromosome, or a user-supplied donor molecule.

Non-homologous end joining (NHEJ): a major DNA DSB repair pathway. Repair is non-template directed, and involves the direct religation of the exposed DNA ends. Repair can result in deletions, substitutions, or insertions at the break site [57]. The error-prone nature of NHEJ, combined with the ability to direct DSBs with sequence-specific nucleases, provides genome engineers with an approach to mutagenize sequences within living cells.

Sequence-specific nucleases: a family of enzymes consisting of meganucleases, zinc-finger nucleases, TALENs, and CRISPR/Cas. All sequence-specific nucleases can be engineered to bind to and cleave a DNA sequence of interest.

Synthetic biology: combines science and engineering to design and construct new biological parts, devices, and systems. The definition also includes the creation and integration of new biological systems.

Trait stacking: also known as gene stacking, this refers to the process of adding two or more genes into a plant genome at the same location.

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Keywords: synthetic biology; sequence-specific nuclease; zinc-finger nuclease; TALEN; CRISPR/Cas; genome engineering.

0167-7799/

 http://dx.doi.org/10.1016/j.tibtech.2014.11.008

Box 1. A spotlight on two ambitious synthetic biology projects

Examples of plant synthetic biology projects include the nitrogen-fixing cereals project (Figure IA) and the C_4 rice project (Figure IB).

By engineering cereals to uptake atmospheric nitrogen, there will be a reduced dependency on inorganic fertilizers. There are two possible approaches for modifying cereals to uptake atmospheric nitrogen: transfer the nodulation signaling pathway from legumes to promote root nodule symbiosis with *Rhizobium* bacteria, or engineer the nitrogenase enzyme to function in plant cells.

Engineering the C_4 photosynthesis pathway into C_3 rice promises to increase yield. One approach to engineering this pathway in rice is to convert the single-cell C_3 cycle into a two-celled C_4 cycle. In this case the initial carbon fixation is catalyzed within mesophyll cells by phosphoenolpyruvate carboxylase (PEPC) forming the four-carbon oxaloacetate from bicarbonate and PEP. Oxaloacetate is then metabolized into malate, and the four-carbon acid diffuses into the bundle sheath cell. There, the four-carbon acid is decarboxylated to provide increased concentrations of carbon dioxide to RuBisCO, which is confined in bundle sheath cells.

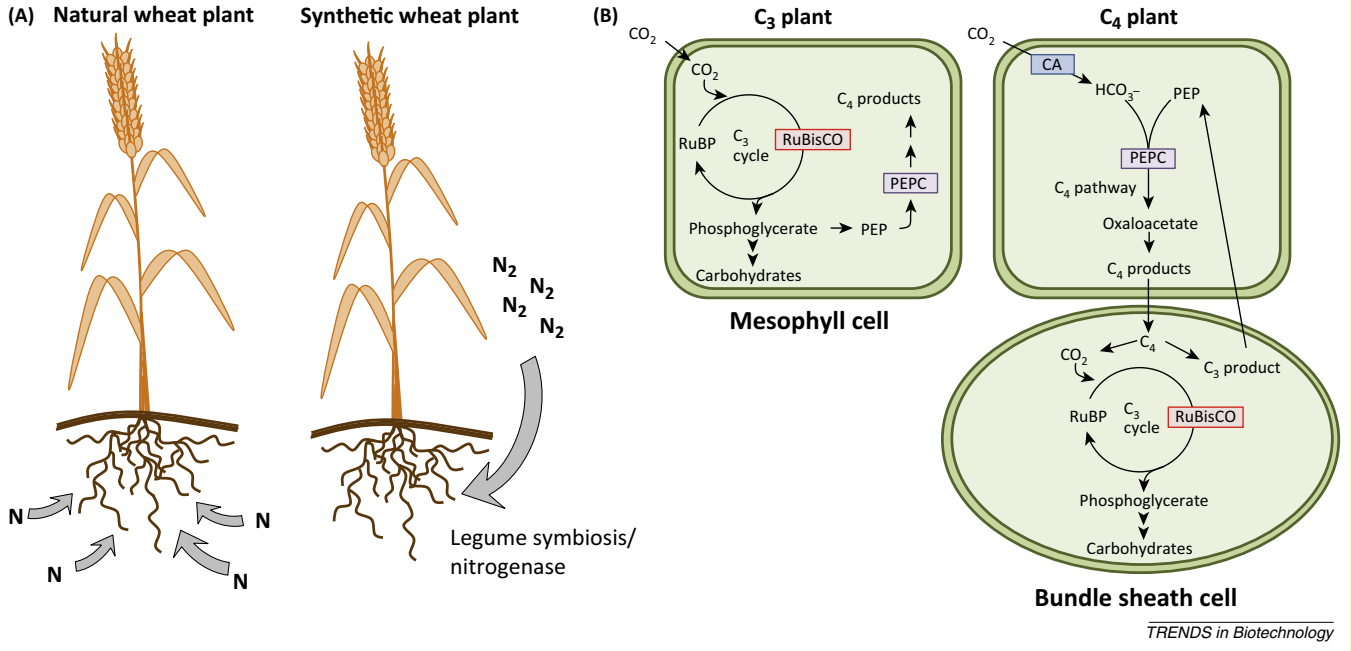

Figure I. Two examples of synthetic biology projects aimed at generating nitrogen-fixing cereals or C_4 photosynthetic rice.

fight off pathogens, and they are not subject to the ethical issues that sometimes limit the use of animal cells. Finally, plants use abundant and inexpensive nutrients (carbon dioxide and sunlight) to produce their primary and secondary metabolites, and their total biomass is enormous: approximately 210 billion tons of plant material are produced each year [4].

Approximately 30 years ago the first plants were generated with novel functions, including herbicide tolerances and insect resistances [5]. These plants were made through transgenesis, in which user-designed DNA was randomly integrated into plant genomes. While this was an important first step in designing plants with novel functions, the past few years have witnessed the emergence of more sophisticated and precise methods for engineering DNA in living cells. When these methods are used to their fullest potential, they can generate any type of modification within plant genomes, ranging from precisely introducing one or more transgenes at a desired locus, to removing unwanted or unnecessary DNA from the host, to accurately controlling expression of host or synthetic genes.

Even by focusing on user-designed plants, the breadth of projects that fall under the synthetic biology term is enormous. Examples of such projects include: (i) modifying cereals, including wheat, to fix atmospheric nitrogen, (ii) redesigning metabolic pathways to increase the yield of secondary metabolites or to generate compounds with enhanced properties, (iii) transferring the C_4 photosynthesis pathway to rice, (iv) modifying the glycosylation pathway in plants to accommodate production of therapeutic proteins, and (v) introducing synthetic signal transduction systems that respond to external cues [6]. A common ground for most synthetic biology projects is the need for standardized genetic parts (e.g., promoters, terminators, genes), and the subsequent need for tools and techniques for modifying plant genomes.

Engineering genomes with sequence-specific nucleases

One method to efficiently and precisely modify plant genomes involves introducing targeted DNA double-strand breaks (DSBs) at a locus of interest. Normally, DSBs are highly toxic lesions, and to preserve the integrity of their genomes, all living organisms have evolved pathways to repair such breaks. In general, plant cells have two main DNA repair mechanisms: non-homologous end joining and homologous recombination [7]. As described in greater detail below, repair by either pathway can be exploited to introduce sequence changes within genomes.