Using synthetic biology to unleash the potential of plants

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Synthetic biology applies the principles of engineering to biology, helping us to design, redesign and build novel biological systems. First applied to microorganisms it has enabled the industrial-scale production of beneficial, bio-based products such as pharmaceuticals, flavourings and textiles. It is now advancing to multicellular organisms. In plants, the field is still in its infancy, yet the potential for plants as light-powered, sustainable biomanufacturing platforms is tremendous.

Plants are the source of a great diversity of biologically active, high-value small molecules. Artemisinin, used to treat malaria, is produced by sweet wormwood; morphine and salicylic acid from poppies and willow bark, respectively, are widely used as painkillers and a number of molecules found in Madagascan periwinkles, Pacific yew trees and species of Euphorbia are used as chemotherapeutics. However, these valuable molecules are often found in low abundance, or are produced in species that are difficult to cultivate. While some molecules can be produced using chemical synthesis, this approach has proven economically unfeasible for many, due to their complexity.

In recent years, metabolic engineering and the application of synthetic biology approaches have enabled the biosynthesis of a number of plant natural products in alternative hosts. Artemisinin has been produced in *Saccharomyces cerevisiae* (baker's yeast) as have flavour molecules such as vanillin (vanilla) and nootkatone (grapefruit). While microorganisms have proven powerful hosts, some molecules are not easily produced in high yields in such distantly related organisms. The development of alternative 'easy-to-engineer' photosynthetic hosts is desirable to improve access to a diverse range of plant metabolites and, potentially, to improve the sustainability of manufacturing, for example by reducing the requirement for plant-based sugars as feedstock for microbes.

Plants: biodegradable, sustainable biofactories

Plants, algae and cyanobacteria capture carbon dioxide from air and utilize solar energy through photosynthesis to produce simple sugars. These are then metabolized into a staggering range of carbon-based products, such as lignin, carbohydrates, lipids and plenty more besides, including many of the highly complex molecules we use as medicines, flavours and fragrances every day.

Plant metabolism is categorized into primary and secondary (or specialized) metabolism (Figure 1). Primary metabolism produces the carbohydrates, lipids and amino acids required for growth and development, while secondary metabolism converts primary metabolic precursors into specialized compounds. These compounds, though not necessarily essential for plant growth and development, are used for processes such as defence and the attraction of pollinators. Over 50,000 different molecules have been identified from plants.

Obtaining commercial quantities of pure compounds from the native plant species in which they are produced can be challenging. A number of different plant natural products used as therapeutics frequently feature on the lists of drugs in short supply compiled by various national and international agencies.

The supply of some compounds has been secured by culturing the cells producing the greatest quantities. For example, paclitaxel is a diterpene alkaloid from Pacific yew trees (*Taxus brevifolia*) used as a chemotherapeutic that binds to and stabilizes microtubules preventing their reorganization during cell division, which leads to cell cycle arrest. In the early days the compound was purified from bark obtained by logging, which killed the trees. Later, it became possible to carry out a partial chemical synthesis from more abundant precursors found in the same species and, more recently, production has become possible by cell culture.

However, armed with genomics and synthetic biology approaches, we are gaining the ability to unleash

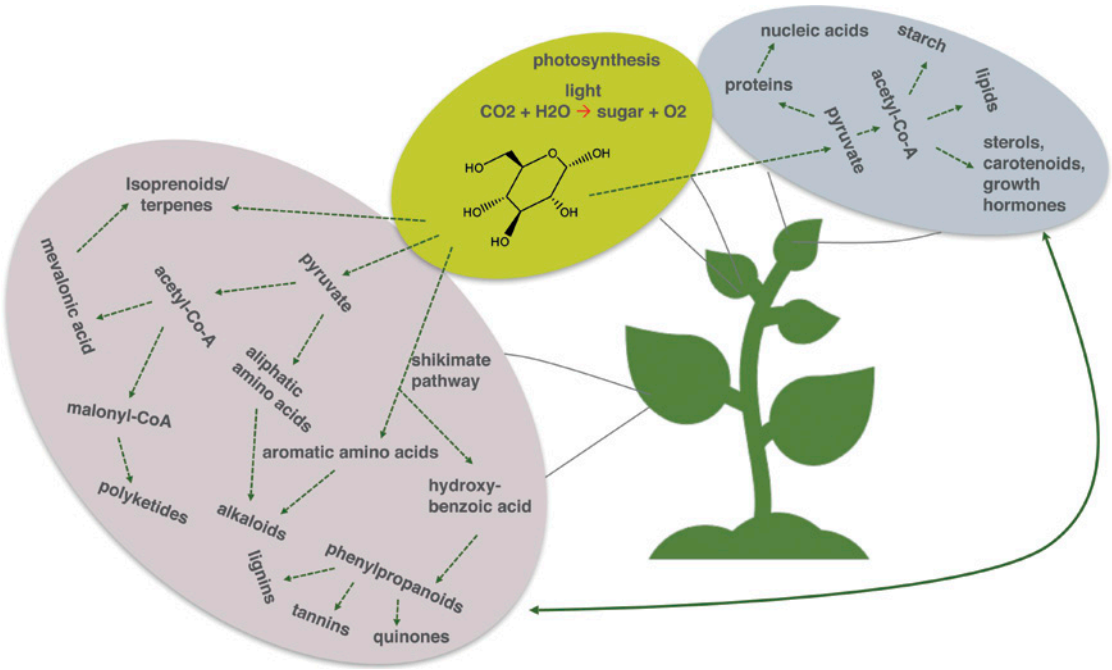

Figure 1. A simplified schematic of carbon metabolism in plants and the formation of primary and secondary metabolites. Atmospheric carbon dioxide is fixed to glucose which is converted to sugars, starch, amino acids, fatty acids etc. These primary metabolite products are fed into pathways to form secondary metabolites such as terpenes, tannins, lignins, alkaloids etc.

the full potential of plants. In recent years, the complete biosynthetic pathways for a number of desirable plant natural products, including vinblastine in Madagascan periwinkle and podophyllotoxin from mayapple, have been identified. These discoveries open the door to production by biomanufacturing in new host cells.

Synthetic biology: application of design-build-test-learn

Contemporary engineering enables computer-based design and accurate prediction of behaviour before any structure is built. For example, it is possible to analyse with significant accuracy the flight characteristics of a new aeroplane before construction has begun. At its most ambitious, synthetic biology aims to enable this for living organisms. By applying the engineering cycle of design-test-build-learn and founding principles such as standardization, automation and abstraction, synthetic biology aims to increase both the scale and predictability of biotechnology.

The phenotype of every living organism is dictated by a combination of the genes coded among the billions of nucleotide bases in DNA and the environment in which that organism lives. While genomics and metabolomics have enabled the identification of genes coding for enzymes responsible for biosynthesis, transferring these genes into new hosts requires regulatory elements that produce the correct quantities of each gene at the correct time. It also requires that proteins fold and function properly, that suitable quantities of metabolic precursors are available, and that the intermediates and end-products of the new metabolic pathways are not destroyed or used to make alternate products by the host cell.

We can now read, write and edit DNA with increasing fluency, facilitating the engineering cycle. Standardized DNA parts encoding regulatory elements can be designed and rapidly assembled in multiple combinations with enzymes that catalyse existing precursors into new compounds. These high-performance pathways can then be introduced to cells. In addition, genome engineering tools such as those derived from bacteria CRISPR/Cas9 systems can be used to remove genes from the host that encode products that use or break down the products of the new pathway. Finally, and perhaps most importantly, the application of quantitative modelling is starting to inform how the design of new DNA and protein sequences inserted into host cells will influence their interactions with each other and with molecules present in the host to affect yield.

Plants as engineerable bioproduction platforms

Plants, historically overlooked as efficient hosts for the engineered production of products other than foods, textiles and building materials, have increasingly been shown to be capable of efficient expression of both therapeutic proteins and small-molecule metabolites. There is significant interest and investment in the use of heterologous plant hosts as bioproduction systems.

Production systems have now been constructed for biomanufacturing in vascular plants on industrial scales. For example, Elelyso (taliglucerase alfa), used to treat type 1 Gaucher's disease, is produced in genetically engineered carrot cells; LeafBio, the commercialization arm of Mapp Biopharmaceuticals, produced the ZMapp therapeutic for ebola in *Nicotiana benthamiana*, a relative of tobacco indigenous to Australia, at Kentucky Processing. Another company, Medicago Incorporated, have constructed a facility for plant-produced vaccines for influenza and other viruses.

Recently, plants have also been used as host cells for the biosynthesis of small molecules. For example, by engineering the expression of regulatory genes together with the expression of genes encoding side chain decorating enzymes, plant cells have been engineered to produce a range of anthocyanin-based pigments (Figure 2). Plants have also been used to produce valuable terpenoids at preparative scales using similar approaches to the commercial-scale production of vaccines. Another useful feature of plants are seeds, which have evolved to cope with adverse environmental conditions and preserve their valuable protein content. These properties have been exploited for the production of proteins such as hirudin, an anticoagulant protein produced in canola (oilseed rape), which has been successfully stored in seeds for three years. A recent attempt to produce an anti-HIV microbicide, recombinant cyanovirin-N (rCV-N) in soybeans also demonstrated the potential of seeds to scale-up production.

To further progress in plants, the synthetic biology engineering cycle that has been so successful in microbial systems is being applied to develop standards as well as synthetic regulatory sequences. For example, modelling approaches have enabled the development of synthetic genetic circuits for plants including: 1) genetic toggle switches, where the expression of genes can be switched on and off using external stimuli, 2) positive/negative feedback loops where a gene product contributes to the regulation of its own expression and 3) genetic Boolean logic gates where the expression level of a gene is dependent on the presence or absence of one or more specific signals (Figure 3).

The range of products that plant production is aiming for is rapidly expanding. One such example is plant-based production of insect sex pheromones. Pheromones are already used and promoted as environmentally friendly alternatives to broad-spectrum pesticides that are believed to have decimated populations of pollinators. Pheromones can be used to disrupt insect mating or trap them. This prevents them from laying eggs that hatch into the destructive larvae that consume the food crops we need to protect. As pheromones are highly species specific, typically emitted by one sex to attract a mate of the same species, their presence has no effect on non target species.

Insects use a great diversity of pheromone molecules. While some can be easily and cheaply manufactured by chemical synthesis, others have complex structures meaning that this approach is economically unfeasible or requires the use of toxic chemicals. Biological production of insect pheromones in plants can offer a greener solution. Pheromones can be engineered in plants by identifying the biosynthetic pathways for the sex pheromones

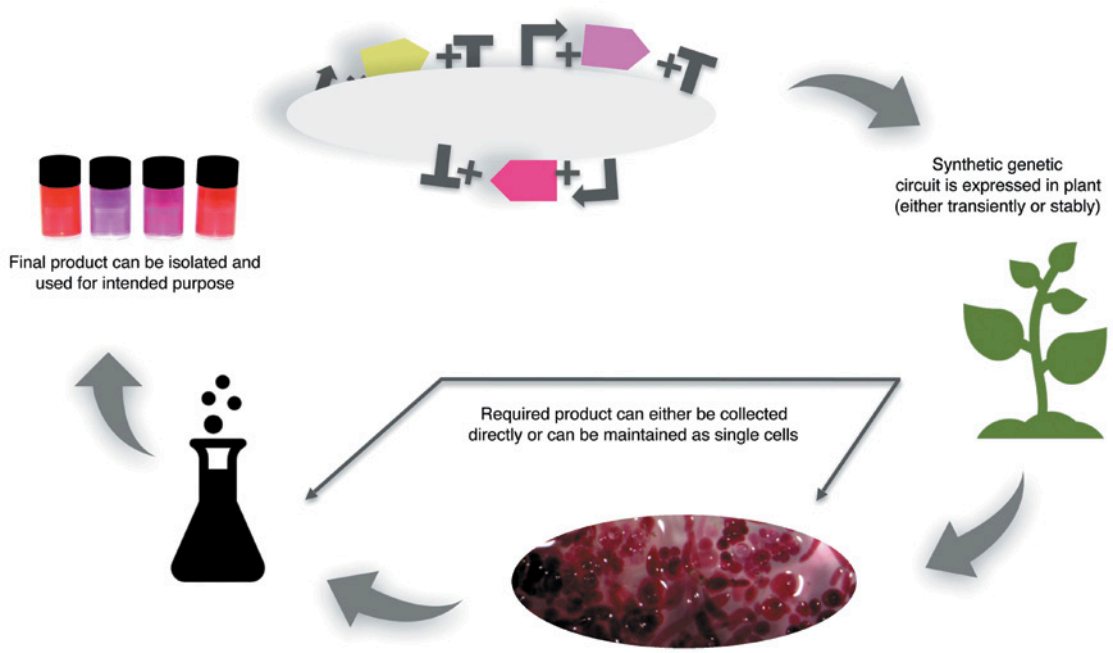

Figure 2. Simplified bioproduction of natural compounds in plants. Genes from different plants or other organisms controlling a specific pathway can be built as a synthetic circuit and transferred to plants. The required product can then be isolated from the plant. Long-term production is possible by growing the transformed plant in cell cultures.

produced in insect pests. By pursuing a design-build-test-learn cycle both the yield and individual components of pheromones can be produced.

A blossoming future

Synthetic biology in plants is in its infancy, yet the potential for plants as biofactories is huge. With at least 50,000 secondary metabolites to investigate, plants provide a vast reservoir of diversity for exploration and use. Furthermore, engineering approaches can be applied to improve crop traits, such as combating pathogens, drought and enhancing nutritional value. Together, synthetic biology, genomics and precision genome editing can be used to improve our harvests and produce entirely novel ones. ■

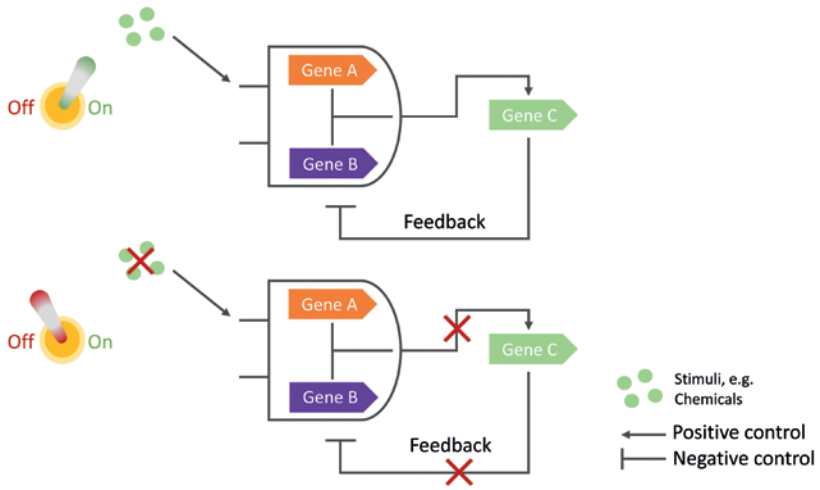

Figure 3. A schematic diagram of a simple AND Boolean logic gate genetic circuit with a feedback loop. Environmental stimuli (light green circles), e.g. a chemical compound, turn on the expression of gene A. Only when gene A and gene B are on at the same time, their output can turn on gene C. The output of gene C can inhibit the expression of gene B which adds a feedback loop in this genetic circuit.

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