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Synthetic genetic circuits in crop plants

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The love affair between crop breeding and genetics began over a century ago and has continued unabated, from mass selection programs to targeted genome modifications. Synthetic genetic circuits, a recent development, are combinations of regulatory and coding DNA introduced into a crop plant to achieve a desired function. Genetic circuits could accelerate crop improvement, allowing complex traits to be rationally designed and requisite DNA parts delivered directly into a genome of interest. However, there is not yet a standardized pipeline from exploratory laboratory testing to crop trials, and bringing transgenic products to market remains a considerable barrier. We highlight successes so far and future developments necessary to make genetic circuits a viable crop improvement technology over this century.

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Introduction

For the purposes of this review, a synthetic genetic circuit is a set of genetic parts, including both coding and regulatory DNA, that are delivered into an organism and together carry out a desired function. The first functional genetic circuits were described in publications almost two decades ago, and comprised a genetic toggle switch and a repression based oscillator, both in *Escherichia coli* [1,2]. Since then synthetic genetic circuits have been delivered into a variety of organisms, including eukaryotes. For example, recently a 10 enzyme metabolic pathway for the production of alkaloids with pharmaceutical applications was described in yeast [3]. In human T-cells, genetic circuits have been constructed that integrate multiple cellular inputs to induce a killing response [4]. In crop plants, genetic circuits may, in the future, allow traits to be designed to order.

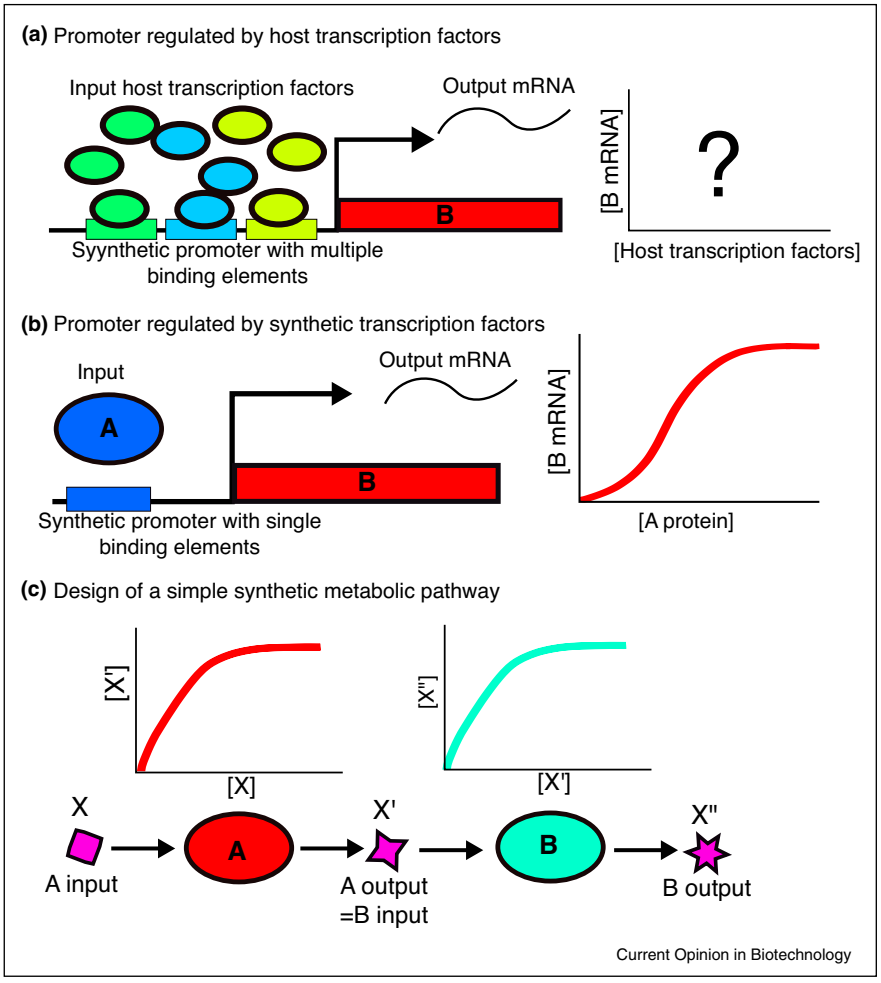
The crop genetic circuit pipeline involves several sequential stages: circuit design, DNA assembly, *in planta* laboratory prototyping, delivery into a crop plant for field trials, and ultimately delivery of the final agricultural product. Of these stages only DNA assembly has been convincingly addressed, with cheap DNA synthesis [5] and reliable methods for scarless multipart assembly of plant gene delivery vectors [6]. In contrast to DNA assembly, there are significant questions remaining at all other stages. How should circuits be designed? How can model plants be effectively used to prototype circuits? How can genetic circuits be efficiently delivered into crop plants? Finally, how can crops containing synthetic genetic circuits be brought to market without resistance from regulators and consumers? This review explores each of those issues in turn.

Approaches to genetic circuit design

Genetic circuit design begins with trait specifications such as increased nutrient level, flower color or pathogen resistance, and translates these into a DNA-based solution. A spectrum of design approaches could in theory be applied to arrive at this solution, from completely random screening of DNA designs through to rational design in a single-step. Random screening is time and labor intensive. Predictive models of plant systems, and accompanying algorithms to reliably generate functional genetic circuit designs, do not yet exist. Even in model microbes, where genetic circuit design is most advanced, software design tools are unable to reliably predict circuit function *in vivo*, although they can help reduce brute-force screening required to build a synthetic genetic circuit by suggesting designs to match user specifications [7]. For now genetic circuit design is likely to combine elements of rational selection of genetic parts with screening to select for desired function. Even without software assistance there are simple design approaches that have proven successful for genetic circuits constructed in plants.

Firstly, circuits should be constructed from *composable parts*. This means that each part should receive a defined input and predictably convert it into a defined output (Figure 1). Selecting parts with compatible outputs and inputs then allows circuits to be built up to achieve complex functions. Plant synthetic promoters composed of cis regulatory elements for endogenous plant transcription factors are commonly used for genetic engineering projects [8] but do not fit the requirements for composable parts (Figure 1a). Since plant transcription factors are often large families with similar binding profiles [9] inputs are often ill defined and the production of outputs, in this case transcriptional units, is not easily predictable,

Figure 1



Composable parts in genetic circuit design. Synthetic promoters containing cis-elements for host transcription factors **(a)** will be regulated by whatever members of that transcription family are expressed in a given cell. Inputs are thus not well defined and the conversion from these inputs to a transcriptional output is hard to predict. By contrast a minimal promoter controlled by a synthetic transcription factor **(b)** has a defined input and the relationship between input transcription factor and output gene expression can be predicted based on initial characterizations. Enzymes are composable **(c)**, converting substrates into products, and are thus often used to create synthetic metabolic pathways. The metabolic pathway here illustrates the principle of composability but is unrealistically simple, excluding, for example, enzyme cofactors. The functions depicted in this figure are illustrative only, but are inspired by Hill equations for transcriptional activation (b), and Michaelis–Menten equations for enzyme kinetics (c).

with behaviour varying according to developmental context [10] among other factors. In contrast, minimal promoters controlled by synthetic transcription factors have defined inputs, and *in vivo* promoter function can, at least broadly, be predicted from earlier characterization (Figure 1b; [11••]). Assuming substrate and product specificity, enzymes fit the requirements of composable genetic parts. They convert substrate X into product X'. Indeed synthetic metabolic pathways make up the largest group of synthetic genetic circuits developed so far in crop plants [12–16] (Figure 1c). While assembly from composable parts may facilitate successful design it is important to note that screening remains a necessary step. Composable parts actually facilitate screening since equivalent input/output conversions can be swapped out until circuit performance matches specifications.

A second enabling design approach is to use *heterologous* genetic parts, meaning those drawn from other organisms or *de novo* designs. As in the comparison of promoters controlled by host or synthetic transcription factors (Figure 1a,b) the use of heterologous parts helps to define inputs and predict outputs. In addition, heterologous parts are more likely to exhibit *orthogonality*, meaning that they do not interact with host components in unexpected ways that may interfere with circuit function. The advantage of heterologous parts and accompanying orthogonality was demonstrated in a comparison of