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Programmable genetic circuits for pathway engineering

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Synthetic biology has the potential to provide decisive advances in genetic control of metabolic pathways. However, there are several challenges that synthetic biologists must overcome before this vision becomes a reality. First, a library of diverse and well-characterized sensors, such as metabolite-sensing or condition-sensing promoters, must be constructed. Second, robust programmable circuits that link input conditions with a specific gene regulation response must be developed. Finally, multi-gene targeting strategies must be integrated with metabolically relevant sensors and complex, robust logic. Achievements in each of these areas, which employ the CRISPR/Cas system, *in silico* modeling, and dynamic sensor-regulators, among other tools, provide a strong basis for future research. Overall, the future for synthetic biology approaches in metabolic engineering holds immense promise.

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Introduction

Synthetic biology is a burgeoning field, with varied roots and a brief, contentious history [1••,2]. The field is poised to provide meaningful technological advancements to metabolic engineering in the form of programmable circuits. These will enable metabolic engineers to exert precise, programmed control over the expression of genes, and help optimize gene expression for a wide variety of metabolic pathways. As a case in point, nitrogen fixation is a potential target process in which multi-input and multi-gene targeting circuits can be applied for advanced metabolic engineering (Figure 1). Nitrogen fixation is a high-value process due to potential agricultural applications, and efforts to engineer nitrogen fixation are already underway [3,4]. Not only is nitrogen fixation an energy intensive and potentially burdensome process, but the central enzyme, nitrogenase, is also irreversibly inactivated by oxygen. Strict regulation of gene expression through multi-input and multi-gene targeting circuits will ensure that genes are only expressed in the absence of oxygen, and when culture conditions are such that the necessary energy can be spared. Furthermore, feedback and feedforward mechanisms can allow cells to dynamically regulate expression levels on the basis of metabolic requirements, preventing wasteful protein synthesis.

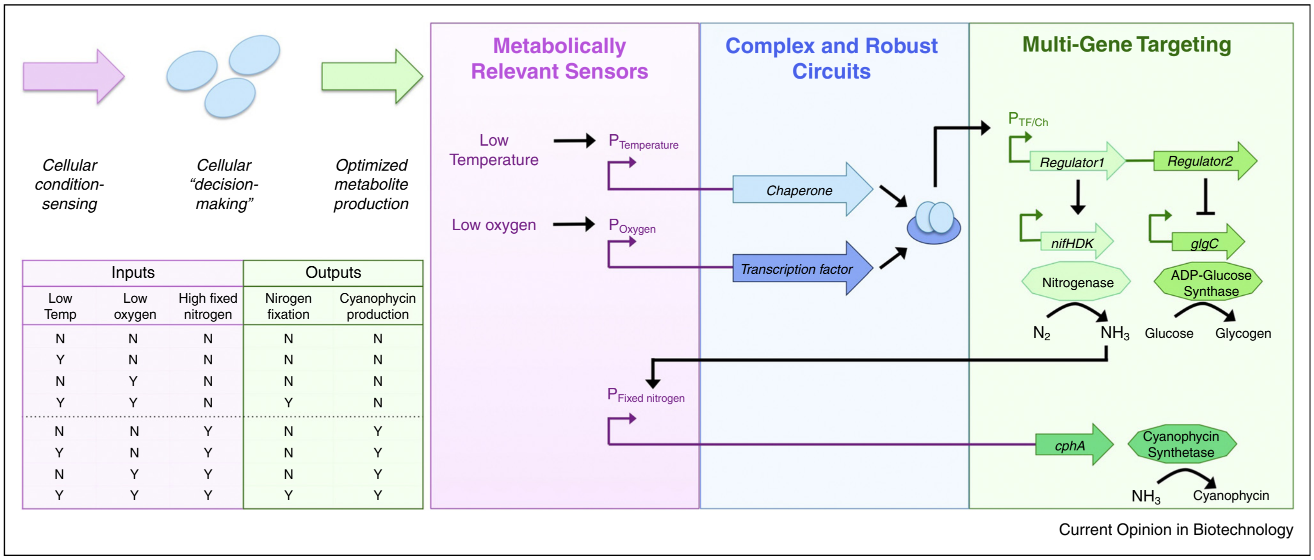
The transition from bench-scale circuit construction [5,6] to full-scale pathway optimization [7] necessitates the development of metabolically relevant sensors, complex and robust circuit behavior, and multi-gene targeting strategies that can provide genome-wide regulation. First, recent progress in the discovery or development of genetic sensors will be discussed, with some examples of the integration of these sensors into simple genetic circuits for metabolic pathway optimization. Next, we will describe achievements in circuit construction, as well as the balance between circuit complexity and robustness. Finally, multi-gene targeting strategies will be described, with a particular focus on applications to metabolic pathways.

Discovery and development of metabolically relevant sensors

Many genetic circuits have utilized inducible promoters adapted from natural systems that respond to chemical inducers. Although such inducible promoters are invaluable research tools, they are not particularly useful for large-scale metabolic engineering applications. Chemical inducers must be added exogenously by the researcher, requiring a ''hands-on'' approach to gene regulation. Additionally, chemical inducers can be expensive, making their use in large-scale reactors cost-prohibitive. Some researchers have tackled this problem by developing promoters that respond to metabolites within a given pathway. This strategy empowers cells to respond to an internal signal, allowing a ''hands-off'' approach, in addition to cutting inducer costs. Furthermore, engineering cells to be more responsive to their internal state can result in improved efficiencies. Several reviews have addressed the topic of the design, discovery, and optimization of such sensors [8–10].

The main challenge that synthetic biologists are currently facing on this front is the construction of a library of high-performing and well-characterized sensors that respond to a variety of cellular signals. Many of these sensors exist naturally. For instance, many sugar-responsive sensors can be mined from existing genomes by identifying genes

Figure 1



Proposed programmable genetic circuit in a metabolic engineering application. Cells will sense their environmental conditions (temperature [17•] and oxygen concentration) and metabolic state (fixed nitrogen availability). These inputs will inform ''decisions'' regarding gene regulation *via* a complex and robust genetic circuit, including the AND gate as pictured [31]. Multi-gene targeting strategies will allow for the simultaneous and orthogonal regulation of distinct gene targets. In this case, low temperature and low oxygen will allow the expression of nitrogenase, an enzyme that can fix nitrogen but is irreversibly inactivated by oxygen. At the same time, glycogen synthesis will be down-regulated to ensure that there is sufficient glucose available to provide energy to the process of nitrogen fixation. Fixed nitrogen will activate the expression of cyanophycin synthase, which will store fixed nitrogen as cyanophycin, and increase flux through nitrogenase. The truth table describes the expected outputs that will result from a given set of environmental and metabolic input signals.

involved in sugar metabolism. Synthetic biologists have made strides in characterizing and modifying these natural sensors to fit the requirements of synthetic circuits [11–13]. Another approach to natural sensor discovery was demonstrated by Dahl *et al.*, wherein comparative whole-genome transcript arrays were used to identify promoters that would respond to farnesyl pyrophosphate (FPP), a toxic intermediate of the isoprenoid pathway [14••]. As an alternative to mining for sensors in the genome, some studies have demonstrated that sensors can be designed *de novo*. Carothers *et al.* demonstrated that a model-driven approach could be used to design ligand-binding RNA devices that respond to metabolic pathway intermediates [15]. Continued work in this field will allow for the development of a well-characterized library of genetic sensors.

In addition to metabolite sensors, environmental sensors have also been developed to respond to environmental signals. A potential application of such sensors is illustrated in the proposed nitrogen-fixing cyanobacterium (Figure 1). Cells growing in photobioreactors often experience overheating during the late afternoon [16]. Down-regulating an energy intensive process like nitrogen fixation during this stressful period, enabled by a heat-repressible expression system [17•], will allow the organism to survive, and is likely to result in a higher overall productivity. In addition, because nitrogenase is irreversibly inactivated by oxygen, including an oxygen sensor in the circuit design will prevent futile gene expression. Coordinated implementation of both oxygen and temperature sensors can ensure that gene expression occurs only under the appropriate conditions, reducing unnecessary protein synthesis.

Metabolically relevant sensors in simple genetic circuits

One benefit of using metabolically relevant sensors to regulate metabolic pathways is that gene expression can be tied to substrate availability. The first engineered system to link a metabolically relevant sensor with a simple circuit used the *glnAp2* promoter to control two genes related to lycopene production [18]. Because the *glnAp2* promoter is activated by high glucose flux, lycopene was produced only when there was excess glucose flux in the cell, increasing lycopene productivity three-fold and reducing waste. This study paved the way for later work in the development of dynamic sensor-regulator systems [19•].

Dynamic sensor-regulators generally use intermediate-sensing systems to ensure that pathway genes are controlled by precursor availability, limiting unnecessary protein production and pushing substrates through the