Leading Edge
Review

Cell

Engineering Cellular Metabolism

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Metabolic engineering is the science of rewiring the metabolism of cells to enhance production of native metabolites or to endow cells with the ability to produce new products. The potential applications of such efforts are wide ranging, including the generation of fuels, chemicals, foods, feeds, and pharmaceuticals. However, making cells into efficient factories is challenging because cells have evolved robust metabolic networks with hard-wired, tightly regulated lines of communication between molecular pathways that resist efforts to divert resources. Here, we will review the current status and challenges of metabolic engineering and will discuss how new technologies can enable metabolic engineering to be scaled up to the industrial level, either by cutting off the lines of control for endogenous metabolism or by infiltrating the system with disruptive, heterologous pathways that overcome cellular regulation.

Introduction

For at least 8,000 years, humans have harnessed microbes to produce fermented foods and beverages. In more recent history, microbes have been used to produce chemicals for a wide range of applications. During World War I, Chaim Weismann developed the acetone-butanol-ethanol fermentation process, which was used for ~50 years to produce acetone and is now being revived for production of 1-butanol. In the 1920s, fermentation of the filamentous fungus *Aspergillus niger* was adapted to generate citric acid, a food and beverage ingredient. During World War II, the same technology was used for industrial scale production of penicillin, the first pharmaceutical produced by fermentation.

The following decades witnessed a dramatic increase in the use of microorganisms to synthesize natural products of pharmaceutical interest, such as antibiotics, cholesterol lowering agents, immunosuppressants, and anti-cancer drugs. Improved performance of classical fermentation processes for such purposes was typically achieved through mutagenesis and screening. For antibiotics in particular, this was an extremely efficient approach, with penicillin production using *Penicillium chrysogenum* boosted by more than 10,000-fold (Thykaer and Nielsen, 2003). Although genetic engineering made it possible to use a more directed approach to improve metabolism, most work focused on the development of cell factories for production of recombinant proteins for use as pharmaceuticals, and today, there are more than 300 biopharmaceutical proteins and antibodies on the market with sales exceeding $100 billion (Langer, 2012).

With the late 1980s and early 1990s came new insights into the complex inner workings of cellular metabolism, fueled by bioinformatics and mathematical modeling methods that allowed quantitative analysis. This enabled specific genetic modifications altering cellular metabolism to be introduced, such that fluxes could be directed toward the product of interest. Thus, the field of metabolic engineering was born (Bailey, 1991; Stephanopoulos and Vallino, 1991; Nielsen, 2001; Keasling, 2010). Now, more than twenty years later, metabolic engineering has been exploited not only to improve traditional microbial fermentation processes, but also to produce chemicals that are currently used as fuels, materials, and pharmaceutical ingredients (Table 1).

Despite the advanced systems and synthetic biology technologies now available for detailed phenotypic characterization of cells and genome editing, developing new cell factories that meet the economic requirements for industrial scale production is still challenging, typically requiring 6–8 years and over $50 million. The reason for this is inherent to the cells themselves. To ensure metabolic homeostasis even when exposed to varying environmental conditions, cells have evolved extensive regulation and complex interactions between metabolic pathways. Redirecting carbon fluxes toward desired metabolites therefore requires modulating the lines of communication in endogenous metabolic pathways or infiltrating the system with disruptive signals that interfere with these regulatory mechanisms. At present, our knowledge of how metabolism is regulated even in simple model cells is limited. As a result, engineering a cell factory involves several rounds of the so-called "design-build-test" cycle, in which a certain metabolic design is implemented and improved through genetic engineering and thereafter tested.

Table 1. Some Success Stories of Metabolic Engineering

Chemical	Application	Cell Factory	Companies
Lysine	feed additive (>1 million tons/year)	*Corynebacterium glutamicum*	Evonik, ADM, CJ, Ajinomoto
1,3-Propanediol	chemical building block, e.g., for production of materials, cosmetics, and food ingredients	*Escherichia coli*	Dupont and Tate&Lyle joint venture
7-ADCA	precursor for the broad-spectrum antibiotic Cephalexin	*Penicillium chrysogenum*	DSM
1,4-Butanediol	chemical building block, e.g., for production of Spandex	*Escherichia coli*	Genomatica
Artemisinic acid	anti-malarial drug	*Saccharomyces cerevisiae*	Sanofi Aventis (process developed by Amyris)
Isobutanol	advanced biofuel	*Saccharomyces cerevisiae*	Gevo, Butamax

Here, we will discuss the principles and current challenges of metabolic engineering, focusing on how metabolism can be engineered for industrial level production of specific chemicals, either through de-regulation of endogenous metabolism or through insertion of heterologous pathways that overcome cellular regulation. We will then discuss how technologies developed in recent years can contribute to the design-build-test cycle and how adding a fourth element to this cycle, namely "learn," can improve the process. Based on implementation of specific metabolic designs, can we gain new knowledge about how metabolism operates and how it is regulated and subsequently use this knowledge for improved design?

Challenges for Metabolic Engineering

Even though metabolic engineering has found applications in optimization of existing processes, much of the current focus is on the development of novel bioprocesses. In the fuel and chemical industry, there is much interest in exploiting the potential of bio-based production for two major reasons: the sustainability factor and the possibility of producing new molecules. Bio-based production of chemicals allows for use of renewable raw materials, such as plant-derived feedstocks like starch, sucrose, cellulose, and lignocellulose that are more sustainable than many traditional chemical processes relying on fossil fuels. Furthermore, replacement of traditional chemical synthesis with bio-based production typically results in reduced environmental footprint in terms of energy usage and emission (Saling, 2005). The key driver for the chemical industry is, however, the production of chemicals that have either better properties than traditional chemicals or chemicals that can find new applications.

The Route for Development of a Novel Bioprocess

Production of a so-called "drop-in" chemical starts with identification of the molecule of interest, followed by determination of whether there exists a metabolic pathway in nature to produce this molecule (Figure 1A). Drop-in chemicals are molecules produced by fermentation instead of from fossil feedstock or other natural sources that are difficult to work with (such as rare plants). In some cases, it is possible to identify a natural producer of the molecule, and this cell factory can then be used for further improvement. If, on the other hand, you want to transfer the biosynthetic pathway to a heterologous host, and if all of the enzymes of the biosynthetic pathway have not yet been identified, heterologous expression requires enzyme discovery as part of the metabolic engineering program, as illustrated for production of artemisinic acid (Ro et al., 2006; Westfall et al., 2012; Paddon et al., 2013) and opioids (Galanie et al., 2015). In some cases, however, it is difficult to identify all the biosynthetic enzymes needed to produce a molecule, and this hinders pathway reconstruction in a heterologous host. For instance, not all the enzymes involved in biosynthesis of the anti-cancer drug taxol have yet been identified (Ajikumar et al., 2010). Improved technologies for DNA and RNA sequencing, bioinformatics, and structure-function predictions have advanced our ability to rapidly identify enzyme candidates for a specific biosynthetic pathway that can subsequently be evaluated for their ability to reconstruct a complete pathway. In case it is not possible to identify a natural producer, chimeric pathways may have to be reconstructed and some of the enzymes may have to be evolved or engineered to have new features.

Traditionally, natural producers were developed for production of the molecule of interest through classical strain improvement. However, with the advent of metabolic engineering, the preferred route for developing a novel bio-process is now through the use of "platform cell factories" (Figure 1A). Examples include *Saccharomyces cerevisiae*, *Escherichia coli*, *Aspergillus niger*, *Bacillus subtilis*, *Corynebacterium glutamicum*, and Chinese hamster ovary (CHO) cells. The advantage of using platform cell factories are numerous: (1) they are very well characterized in terms of genetics and physiology; (2) it is easier to obtain product approval by governmental organizations if they have been used for production of a range of products already; (3) many tools for genome editing are available; and (4) many gene expression tools are available, e.g., plasmids, promoters, and terminators. Each of the above mentioned cell factories have specific advantages. For example, *A. niger* and *B. subtilis* have very efficient protein secretion and are therefore widely used for production of industrial enzymes, while CHO cells are well suited for production of glycosylated proteins to be used as pharmaceuticals. For fuels and chemicals, there is an increasing focus on use of *S. cerevisiae* and *E. coli* as platform cell factories, with *C. glutamicum* as an attractive third choice. To produce a molecule of interest, the biosynthetic pathway for the molecule is reconstructed in the platform cell factory, resulting in establishment of a proof-of-principle strain (Figure 1B). Generally, this strain can be patented and represents a key milestone in the development of a novel bioprocess.