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Reevaluating synthesis by biology

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The two cornerstones of synthetic biology are the introduction of the new technology of chemical DNA synthesis and its subsequent emphasis on the use of standardized biological parts in the construction of genetic systems aimed at eliciting of desired cellular behavior. A number of high-impact applications have been proposed for this technology, notable among them being the biological synthesis of valuable compounds for chemical or pharmaceutical use. To this end, synthetic biologists propose assembling metabolic pathways *in toto* by combining genes isolated from a variety of sources. While pathway construction is similar to approaches established long ago by Metabolic Engineering, the two methods deviate significantly when it comes to pathway optimization. Synthetic biologists opt for gene-combinatorial methods whereby large numbers of pathways, comprising several combinations of genes from different sources, and their mutants, are evaluated in search for an optimal pathway configuration. Metabolic engineering, on the contrary, aims to optimize pathways by tuning the activity of the intermediate reaction steps. Both, rational methods based on kinetics and regulation, as well as combinatorial methods, typically in this order, are used to this end. We argue that a systematic approach consisting of fine-tuning the properties of individual pathway components, prominently enzymes, is a superior strategy to searches spanning large genetic spaces in engineering optimal microbes for the production of chemical and pharmaceutical products.

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A new paradigm for pharmaceutical manufacturing

Despite its brief history, synthetic biology is already making significant contributions that bring biology at the forefront of chemical and pharmaceutical manufacturing [1], especially the production of high-valued chemicals and pharmaceutical products [2••]. Considering that the therapeutic properties of most medicinal plants are attributes of the unique bioactive molecules that they synthesize, that of the 877 small-molecule New Chemical Entities (NCEs) that were introduced between 1981 and 2002, as many as half were either natural products, semi-synthetic natural product analogs or substances derived from natural products [3], and that synthetic biology offers a platform to efficiently express the biosynthetic pathways producing these molecules in microbes, it is not surprising that developments in the field have elevated the status of synthetic biology to that of an enabling technology for the synthesis of valuable compounds.

Engineering microbial metabolism for natural product synthesis

Several factors make the synthesis of bioactive molecules by microorganisms grown on cheap sugar feedstocks in readily scalable bioreactors a compelling proposition, notably, the prohibitively low yields and much higher costs associated with rival methodologies such as *de novo* chemical synthesis or extracting them from their natural hosts. In addition, the metabolic pathways synthesizing bioactive molecules in plants have quite a few genes in common, implying that such pathways can be modularized into homologous and variable operons. Metabolic engineers have also been endeavoring to engineer microorganisms to express plant metabolic pathways, and have been doing so for quite some time now [4–6]. In these efforts, they aimed to alter the metabolic landscape within the host cell by manipulating components from all strata of the metabolic network [7–10], drawing on principles and techniques from diverse practices, such as genetic engineering and molecular biology for pathway construction and control, and enzyme engineering and (bio)chemical reaction engineering for pathway modulation and efficient operation. The advent of DNA chemical synthesis has further facilitated the introduction of genes from different sources in the construction of such pathways, each suitably codon-optimized for efficient expression.

Once the desired heterologous pathway has been inserted into the host and it has been suitably harmonized with the native metabolism of the cell, those metabolic components that have been characterized as crucial determinants of pathway performance – be it a single gene, enzyme, or metabolite, or a combination of several genes, proteins, and metabolites – are suitably manipulated to elevate the throughput of the heterologous pathway while ensuring that cellular homeostasis and well-being are not adversely affected [7,11]. For example, interventions such as expressing more copies of the enzyme that

catalyzes a rate-limiting step in the heterologous pathway, curtailing the activity of native enzymes that compete with heterologous enzymes for precursors, enhancing the throughput of cofactor synthesizing pathways, and toggling with the activity of a local or global regulator to improve pathway performance, perhaps even disconnecting the regulatory loop entirely if it is detrimental to pathway flux, have been staple techniques in metabolic engineering. Factorial optimization of the parameters that control gene expression might also be required in order to determine the conditions that maximize product formation, and the parameters that are generally varied include strength of expression, gene position within the operon, multiplicity of expression, and mode of expression *viz.* chromosomally or plasmid-based. Occasionally, the source of the gene and its sequence itself may be modified.

The aforementioned method will typically optimize a pathway within the constraints of available knowledge about its kinetics and regulation. As there are many factors yet to be identified that have the potential to impact a pathway's throughput, pathway optimization has benefited by combinatorial approaches whereby the impact of distal genes, transcription factors, or global regulators is assessed with respect to their effect on the function of the pathway. However, since the likelihood of exclusively random methods converging to the specific pathway configurations that optimally combine kinetic and regulatory constraints of the pathway's intermediate steps is quite slim, such combinatorial methods are seldom used independently, and usually follow rational pathway construction and optimization.

The synthetic biology route to natural product synthesis

Greatly aided by the progressively diminishing costs and marked improvements in ease and efficiency of nucleotide synthesis [12–15], synthetic biologists have been assembling evermore elaborate genetic circuits [16–21] with complexities that comfortably exceed those of circuits that can be constructed using recombinant DNA techniques. The rich collection of gene sequences encoding potentially useful chemical transformations, courtesy of metagenomics [22,23], coupled with the ability to rapidly synthesize and vary them, paved the way for synthetic biologists to finally take up natural product and fine chemical synthesis.

It is quite common for the genes encoding similar functions, albeit in different organisms, to exhibit considerable sequence homology, and, contrarily, organisms from similar biotic niches could possess dissimilar genes demonstrating similar functions. Regardless, one can be certain that metagenomics libraries can include multiple variants of a gene. Perhaps swayed by the vast assortment of genetic sequences that they possess, synthetic biologists axiomatically resort to merely varying either the source or the sequences of the pathway's genes to improve product titers. For example, if a pathway comprised 4 genes, each with 3 unique sources, a minimum of 4^3 variants of the expression vector could be rapidly constructed (using massively parallelized nucleotide assembly platforms) and tested for production efficiency following transformation into a suitable host.

In addition, as was demonstrated in a recent study on lycopene synthesis in *E. coli* [24•] that attracted considerable publicity, the list of variants could also be increased by randomly mutating nucleotides at any location within the operon. The satisfactory results obtained by this high-throughput genome engineering and screening methodology have prompted synthetic biologists to hypothesize that the capability to rapidly synthesize genes from different sources and subsequently evaluate the landscape of all possible combinations of such genes using high-throughput screening technologies is sufficient for pathway optimization. It is implicitly assumed that the influence of the gene on the pathway is paramount over all other cellular phenomena, and that the improvements achieved by merely assimilating genes from different sources compare favorably to, if not outdo improvements brought about by using conventional metabolic engineering strategies.

Critique of the synthetic biology approach for pathway optimization

The combinatorial form of synthetic biology outlined earlier attempts to optimize pathways by constructing and assessing vast numbers of candidates each comprising a different combination of genes from a pre-selected set. The underlying assumption is that one of these gene combinations will express enzymes with properties that are optimal for efficient functioning of the pathway. The key question, then, is how likely is it for an optimal pathway configuration to be deduced by simply altering the sources of the genes of the intermediate steps. In its rational form, synthetic biology seeks to replicate fundamental cellular processes or even create new functions by constructing elements of control using standardized genetic parts whose activities and interactions within the cell can be accurately modeled [25–28]. A quantitative understanding of cellular processes is then developed by comparing model predictions with emergent cellular behavior. As such, synthetic biology is a 'bottom-up' discipline that resorts to systematic reconstruction of parts and variants of genetic elements to study their behavior and possibly infer their influence on the behavior of the entire system. In this case one should ask how efficient this method can be in optimizing pathway yield and throughput–the key determinants of microbe engineering.

The above constitutes a significant deviation from the approach followed by metabolic engineering [7,11]. The