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Research review paper

Impact of synthetic biology and metabolic engineering on industrial production of fine chemicals

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ABSTRACT

Industrial bio-processes for fine chemical production are increasingly relying on cell factories developed through metabolic engineering and synthetic biology. The use of high throughput techniques and automation for the design of cell factories, and especially platform strains, has played an important role in the transition from laboratory research to industrial production. Model organisms such as *Saccharomyces cerevisiae* and *Escherichia coli* remain widely used host strains for industrial production due to their robust and desirable traits. This review describes some of the bio-based fine chemicals that have reached the market, key metabolic engineering tools that have allowed this to happen and some of the companies that are currently utilizing these technologies for developing industrial production processes.

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1. Introduction

Cell factories created by engineering metabolic pathways are capable of converting renewable feed-stocks into fuels, chemicals, food ingredients and pharmaceuticals (Keasling, 2010). With increasing climate change awareness alternative transportation fuels are needed. Furthermore, many food, pharmaceuticals and cosmetic ingredients are extracted from plants where seasonal dependent growth can cause supply depletion and extraction methods can be expensive. There is therefore much interest in developing cellular biocatalysts to produce direct replacements for specific chemicals as well as new advanced bioproducts that have properties superior to existing products. Many companies, both biotech and traditional chemical companies, are now translating research successes from both academic and industrial groups to industrial processes. These ventures are motivated by consumer demand for chemical products that are environmentally friendly, less expensive, and possess superior properties compared with those generated by traditional chemical synthesis. Advances in industrial biotechnology and bioengineering over the last two decades and several successful implementations of novel industrial processes have led to significant growth of the field of so-called white biotechnology. Continued advances in DNA synthesis, synthetic biology, and systems biology will only encourage further interest in using genetically engineered cell factories for production of many different chemicals.

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Cell factories are designed and assembled using metabolic engineering and synthetic biology. Tools from these two fields can be used to redirect fluxes towards desired metabolites. The two fields share the same basis in bioengineering with the use of quantitative, model-driven methods for predicting cellular phenotypes (Nielsen et al., 2014), and a common in silico to in vivo approach. Metabolic engineering involves enhancing or redirection of flux through metabolic pathways by making genetic modifications that alter the activity of enzymatic reactions. Genetic modifications include deletion of genes, replacement of gene expression signals, and/or introduction of recombinant DNA cassettes encoding foreign enzymes. Metabolic engineering also includes a detailed analysis of the metabolic pathways to identify targets for manipulation (Nielsen and Jewett, 2008; Ostergaard et al., 2000). These strategies often comprise elimination of unwanted activities, increasing activity at flux controlling steps, and introduction of irreversible reactions to drive the flux in desired directions. Synthetic Biology is the study of how to perform these manipulations in a quantitatively predictive way and how engineering principles can be applied to the design and construction of biological systems. A pillar of synthetic biology is the use of assembly standards for assembling genetic materials. This approach is adapted from other engineering disciplines such as electrical engineering wherein complex systems can be built by combining separate, well-characterized parts. The scope of synthetic biology has grown from construction of codon optimized genes to assembling a complete synthetic genomes (e.g. Mycoplasma "Synthia" (Gibson et al., 2010)), assembling a designer eukaryotic chromosome (Annaluru et al., 2014) and incorporation of new synthetic nucleotides (Malyshev et al., 2014) to increase the information content. The synergy between the two fields will be further exploited to advance research in pathway engineering. However, the two fields can be distinguished as metabolic engineering being a top-down approach, i.e. retrofitting of the metabolism of a cell factory, and synthetic biology being a bottom-up approach, i.e. the reconstruction of a new synthetic cell, is considered (Nielsen et al., 2014).

The performance of cell factories is typically evaluated through three chemical production metrics: titer, rate (a.k.a. productivity), and yield (TRY). In addition, a high performance cell factory should have the qualities needed to thrive in an industrial setting including high osmotic tolerance, broad pH tolerance, and minimal impact on the environment (i.e. generally regarded as safe—GRAS). The yeast *Saccharomyces cerevisiae* meets most of these criteria, and it is therefore widely used as a cell factory for production of food and beverages. Yeast has been in the service of humans since the neolithic period. *S. cerevisiae* has thus been accepted for industrial chemical production and has been assigned to GRAS status. *S. cerevisiae* has also a low risk of contamination due to its low pH tolerance. Homologous recombination, which also occurs to a high degree in yeast, allows for incorporation of genetic fragments into the genome resulting in a more stable strain. *Escherichia coli* is another well-studied cell factory. This model Gram-negative prokaryote is easy to genetically manipulate, an enormous knowledgebase is available, and has been used to develop many of the underlying principles of synthetic biology and metabolic engineering. *E. coli* has been used to produce chemicals commercially and remains a highly studied system in the academic community. Success with model organisms and the difficulties encountered in conferring complex traits to them has motivated interest to develop cell factories from non-model microorganisms that possess desired abilities. For example, photosynthetic and methanotrophic organisms are being developed as cell factories to gain the advantage of using CO_2 or natural gas as carbon source. Adaptation of synthetic biology tools and metabolic engineering strategies for these and other organisms will enable the deployment of future cell factories.

2. From system understanding to design of function

Over the past decades our knowledge about biological systems has increased dramatically. To a large extent this is due to the many techniques that have arisen, not only from biology, but from physics and mathematics and that have proven useful in the service of biology. This is illustrated in Fig. 1, summarizing major technologies and tools in a historical context for the past 20 years combined with upcoming trends. During the past 20 years, technologies have enhanced the scale of bioengineering efforts from individual genes and gene products, through pathways and traits, to complex systems encoded by genome scale DNA—including organisms. Technologies targeting small scales have matured, but much remains to be learned when it comes to designing, constructing, and modifying chromosomes and entire genomes. The technique that has influenced molecular biology the most is the invention of Polymerase Chain Reaction (PCR) in the 1980s (Mullis et al.,

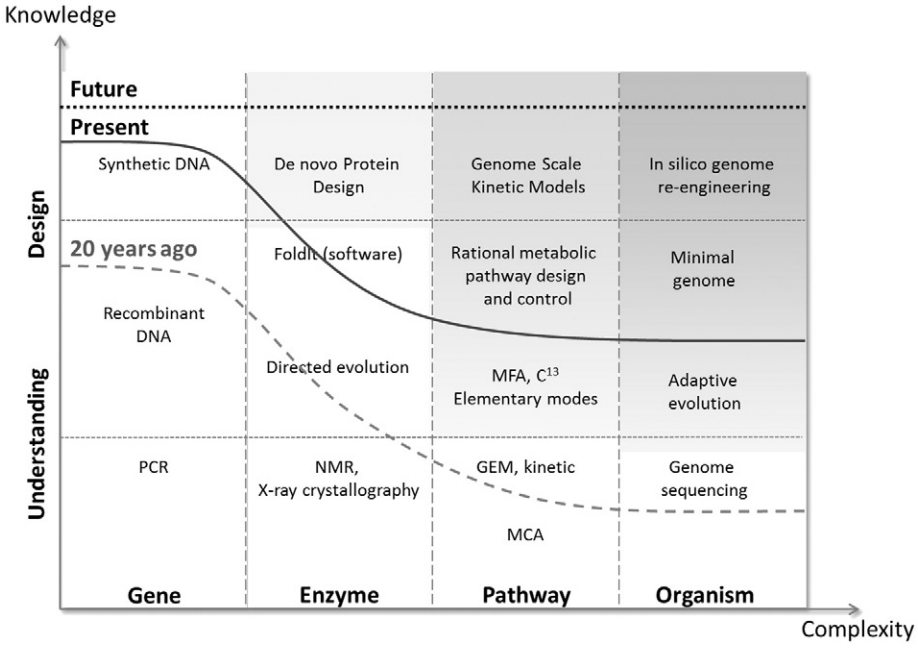

Fig. 1. The graph of knowledge and complexity. At present, we have reached a level of understanding, compared to 20 years ago, that allows us to design parts in a system but we lack a systematic overview to fully understand what makes a system function appropriately.