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Genetic engineering of host organisms for pharmaceutical synthesis

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Pharmaceutical production hosts may be derived from almost any organism, from Chinese Hamster Ovary (CHO) cell lines to isolated actinomycetes. Each host can be improved, historically only through adaptive evolution. Recently, the maturation of organism engineering has expanded the available models, methods, and tools for altering host phenotypes. New tools like CRISPR-associated endonucleases promise to enable precise cellular reprogramming and to access previously intractable hosts. In this review, we discuss the most recent advances in engineering several types of pharmaceutical production hosts. These include model organisms, potential platform hosts with advantageous metabolism or physiology, specialized producers capable of unique biosynthesis, and CHO, the most widely used recombinant protein production host. To realize improved engineered hosts, an increasing number of approaches involving DNA sequencing and synthesis, host rewriting technologies, computational methods, and organism engineering strategies must be used. Integrative workflows that enable application of the right combination of methods to the right production host could enable economical production solutions for emerging human health treatments.

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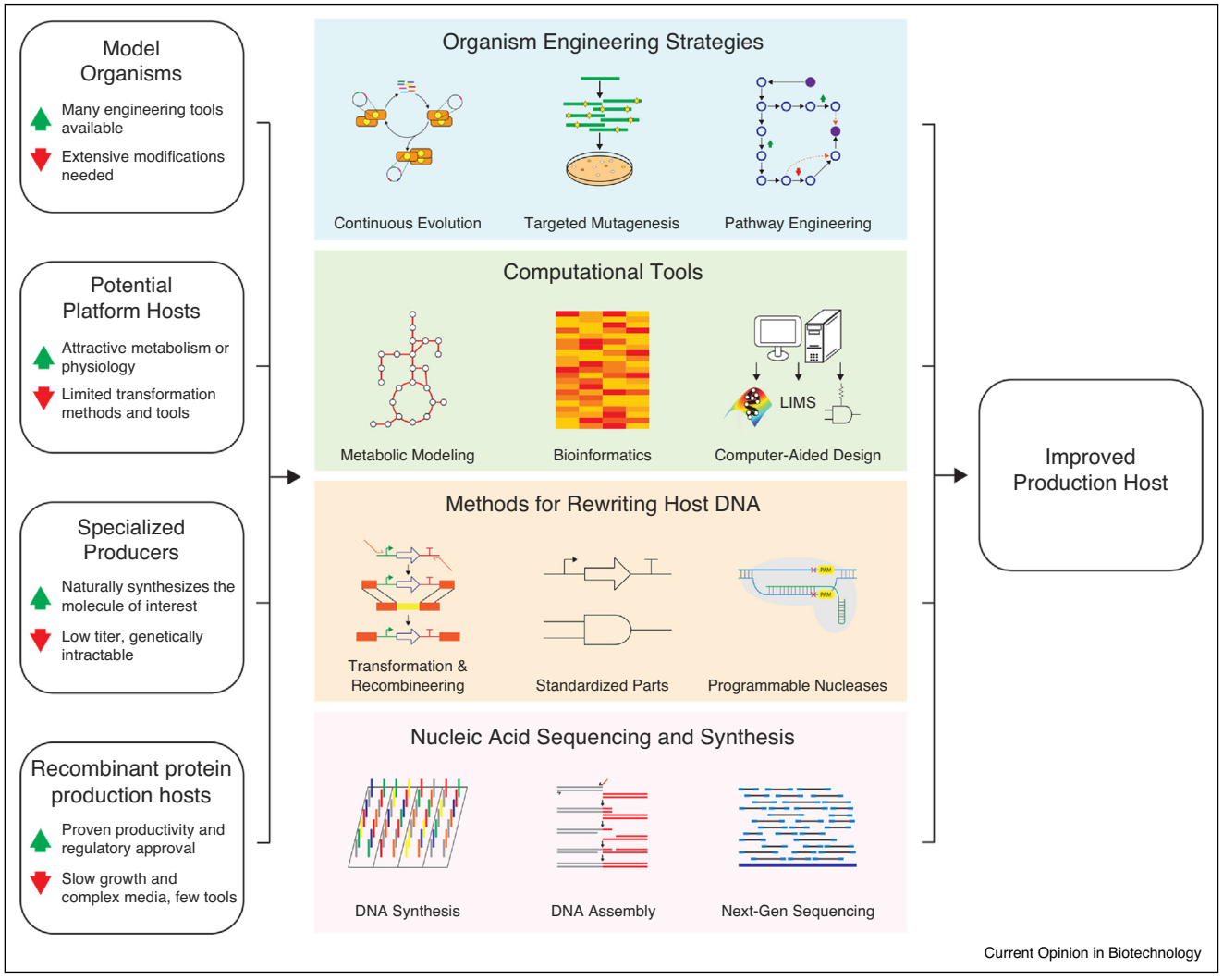
Introduction

Pharmaceutical production processes are under constant economic and regulatory pressure to improve yield, titer, productivity, and purity. Whether producing a therapeutic protein or small molecule, improvements are made by a combination of modifying the host organism, tuning operating conditions, and optimizing unit operations. Yet, most current industrial organisms were established before the maturation of genetic engineering, thus many new approaches in systems biology, metabolic engineering, and synthetic biology remain underutilized in production hosts.

New approaches arise from advances in nucleic acid sequencing and synthesis, rewriting host DNA, computational methods, and organism engineering strategies (Figure 1). Next-generation sequencing (NGS), recently reviewed by McCombie and colleagues [1•], and DNA synthesis, recently reviewed by Kosuri and Church [2], are foundational to engineering biology. NGS and synthesis inform methods to rewrite host DNA, like designing and debugging synthetic parts [3•,4], or constructing and screening CRISPR-based gene editing libraries [5,6]. Rewriting host DNA also requires effective genetic transformation, and new methods like microfluidic electroporation could boost efficiencies or make new organisms transformable [7•,8]. Computational methods are also increasingly required for effective host engineering, enabling genome-scale metabolic models [9•], bioinformatics and systems biology [10], combinatorial pathway optimization [11], laboratory information management software (LIMS) [12•], and automated genetic design [13••]. Breakthroughs in the above areas drive improvements in engineering strategies like model-guided Multiplex Automated Genome Engineering (MAGE) [14••,15], targeted mutagenesis with complete saturation mutagenesis libraries [16], and pathway engineering [17,18••]. Coordinated application of these new tools can simplify and enable engineering across the tree of life, from the newest isolates to the most thoroughly studied model organisms.

As new approaches increase the number of recombinant organisms, host selection must be carefully considered. Hosts may be selected from model organisms, potential platform hosts, specialized producers that make a unique natural product, or recombinant protein production hosts. Each host type has different advantages. Model organisms, such as *Escherichia coli* and *Saccharomyces cerevisiae*, are robust, well-studied, and may be programmed and rewired on a scale not yet possible in other organisms. Potential platform hosts are genetically tractable organisms with attractive metabolic or physiological properties, such as the fast-growing *Vibrio natriegens* [19]. Specialized producers, like strains of actinomycetes and filamentous fungi, can make unique natural products. Recombinant production hosts, such as Chinese Hamster Ovary cells (CHO), have superior posttranslational modification

Figure 1



The landscape of host engineering. Different host types are now accessible with modern engineering methods, and each host has different strengths. To improve each host, workflows that integrate nucleic acid sequencing and synthesis, molecular biology, gene editing, systems biology, synthetic biology, and metabolic engineering are needed. Together, these methods comprise the toolbox for organism engineering. New applications and innovations in each of these fields, from sequencing to predictive models, can change the value proposition for using a particular host. Successful host choice coupled with host engineering can yield improved pharmaceutical production hosts.

profiles, a history of regulatory approval, and an existing manufacturing infrastructure [20]. The strength of one host type is often the weakness of another: model organisms do not make needed posttranslational modifications and do not naturally possess needed biosynthetic pathways, potential platform hosts have limited tools and parts, specialized producers are not genetically tractable and lack tools, and CHO grow slowly relative to bacteria and require costly media formulations.

Looking to the future, with myriad possible hosts and methods available to achieve pharmaceutical production, larger integrated workflows are needed, like the 'living foundries' that are transforming synthetic biology. The foundry approach is not focused on any single host, method, or product. Rather, effort is focused on selecting the right host and the right method for the desired product. To this end, this review discusses recent advances in methods that could influence host selection for industrial pharmaceutical production. Approaches in each host type are highlighted, from applying existing methods in different organisms to novel tools for alternative host engineering. Particular emphasis is placed on the latest CRISPR-associated endonuclease technologies, as these have broadly transformed engineering in all hosts. Emphasis is also placed on advances in CHO engineering, the most widely used production host for recombinant therapeutic proteins.