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Systems biology solutions for biochemical production challenges

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There is an urgent need to significantly accelerate the development of microbial cell factories to produce fuels and chemicals from renewable feedstocks in order to facilitate the transition to a biobased society. Methods commonly used within the field of systems biology including omics characterization, genome-scale metabolic modeling, and adaptive laboratory evolution can be readily deployed in metabolic engineering projects. However, high performance strains usually carry tens of genetic modifications and need to operate in challenging environmental conditions. This additional complexity compared to basic science research requires pushing systems biology strategies to their limits and often spurs innovative developments that benefit fields outside metabolic engineering. Here we survey recent advanced applications of systems biology methods in engineering microbial production strains for biofuels and -chemicals.

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Introduction

One of the key challenges in the 21st century is to identify technical solutions for the transition away from a petrochemical-based economy. The production of chemicals and fuels from renewable feedstocks in a commercially and ecologically sustainable fashion is a central component of these solutions [1]. A handful of bio-based chemicals and fuels have already been commercialized for industrial scale production including 1,3-propanediol [2], succinic acid [3], and 1,4-butanediol [4]. However, despite microbial fermentation-based production offering multiple advantages over current petrochemical processes, its full implementation has been hampered by difficulties in reaching cost-effective yields from low cost feedstocks [5].

Metabolic engineering offers a systematic workflow for rational cell factory development by overexpression of pathway genes, elimination of byproducts, balancing of cofactors and increasing precursor supply among other approaches [6,7]. Recent advances in the field of synthetic biology, such as the development of the CRISPR/Cas9 system [8,9], and other genome editing tools, have increased the pace and ease with which microbial cell factories can be built [10–12]. However, the number of obvious gene targets for optimization is limited, and genetic manipulations often lead to unintended effects due to complex genotype–phenotype relationships [6]. Facilitated by the emergence of high-throughput technologies like next-generation sequencing and quantitative proteomics, systems biology offers several methods to unravel complexity of microbial metabolism and physiology.

The scope of systems biology is to investigate biological systems in a holistic manner to elucidate the mechanisms underlying the cellular behavior in contrast to the classic reductionist approaches where single elements of the system are studied in detail. Similarly, metabolic engineering requires, in addition to manipulation of single enzymes and pathways, also engineering of the interactions between the target pathway and endogenous metabolism [6]. In the field of systems biology, quantitative workflows have been developed in recent years to study responses of microorganisms to relatively simple environmental and genetic changes [13•,14], together with data- and model-driven approaches for predicting phenotypes [15,16]. These workflows can now be extended to engineered cell factories to understand effects of complex manipulations and to design more robust and efficient production organisms.

Here we review some of the most recent applications of systems biology tools for metabolic engineering of microorganisms for sustainable production of chemicals with special focus on non-native biofuels and bulk chemicals. We will focus on three particular technology platforms that have demonstrated impact in metabolic engineering: omics data collection and analysis, genome-scale models (GEMs) of cellular processes, and adaptive laboratory evolution (ALE). Indeed, the integration of omics and computational techniques together with the recent

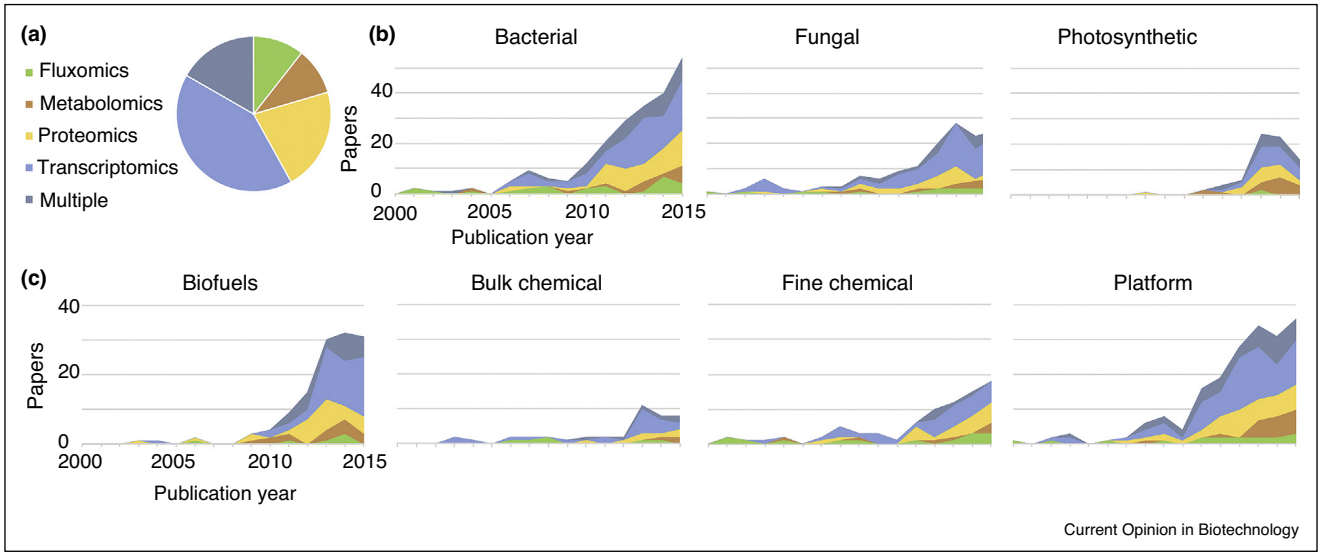
possibility to screen, select and fine-tune cellular responses [17,18] hold promise to speed up the systems metabolic engineering approach. In this context, GEMs offer a useful framework for interpretation of collected data as well as formulation and assessment of potential engineering strategies. The application of ALE for systems-level optimization of host robustness and biochemical production, and the subsequent investigation of causal mutations by omics and computational analysis, allows for simultaneous strain improvement and identification of potential targets for further engineering.

Trends in omics characterization for metabolic engineering

The use of the four major omics technologies (transcriptomics, proteomics, metabolomics, and fluxomics) applied to characterize cell systems behavior has increased rapidly in metabolic engineering-related publications since 2010 (Figure 1). This increase has been brought about both by growth of the metabolic engineering and biofuel fields as well as improvements in omics technologies. Among different omics methods, the development of the quantitative RNA sequencing method has made transcriptomics by far the most commonly used methodology followed by proteomics and in particular targeted pathway-oriented proteomics (Figure 1b). The use of metabolomics and fluxomics (typically ^{13}C-based) is still relatively rare in metabolic engineering studies most likely due to both incomplete coverage of metabolites/fluxes, and challenges and/or costs in experimental implementation. The majority of the metabolic engineering studies using omics data focus on common biofuels that are produced natively (*e.g.*, ethanol, *n*-butanol or fatty acids) or on platform strains without aim to produce a particular product (Figure 1c). Studies using omics technologies characterizing strains making non-native fine or bulk chemicals are surprisingly rare despite well-documented ability of omics methods to discover potential bottlenecks in engineered strains [19].

Recent years have seen the emergence of multi-omic characterization studies that often also incorporate a modeling component to study either platform or production strains. Examples of such studies targeting production strains include identification of bottlenecks in terpenoid production in *Escherichia coli* [20••], 3-hydroxypropionic acid production in baker's yeast [21], and L-lysine production in *Corynebacterium glutamicum* [22]. For platform strains, such studies have included comparisons of multiple possible wild type host strains [23••], in depth characterization of less-well-studied production hosts [24], and determination of effects of major flux re-routing in central metabolism [25]. Multi-omic characterization has also become one of the key tools in identification of mechanisms of adaptation in ALE studies targeting either general stress or product tolerance [26••,27••]. In recent years, standard omics data types are increasingly complemented by genome-wide screening of knock-out or knock-down libraries using, for example, transposon insertion sequencing [28,29] or

Figure 1



Trends in the use of four major omics technologies in metabolic engineering and biofuel publications since 2000. The list of over 500 publications was collected by comprehensive literature searches performed on scopus using terms in title, abstract and keywords followed by manual curation. Genomics was excluded due to the high number of publications using this technology. **(a)** Overall distribution of publications by omics data type used. **(b)** Publication trends as a function of time categorized by type of host organism (heterotrophic bacterial host, fungal host or prokaryotic or eukaryotic photosynthetic host) and omics technology used. **(c)** Publication trends as a function of time categorized by the type of study (biofuel, bulk chemical, fine chemical, platform strain) and omics technology used.