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Review

Metabolomics methods for the synthetic biology of secondary metabolism

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ABSTRACT

Many microbial secondary metabolites are of high biotechnological value for medicine, agriculture, and the food industry. Bacterial genome mining has revealed numerous novel secondary metabolite biosynthetic gene clusters, which encode the potential to synthesize a large diversity of compounds that have never been observed before. The stimulation or "awakening" of this cryptic microbial secondary metabolism has naturally attracted the attention of synthetic microbiologists, who exploit recent advances in DNA sequencing and synthesis to achieve unprecedented control over metabolic pathways. One of the indispensable tools in the synthetic biology toolbox is metabolomics, the global quantification of small biomolecules. This review illustrates the pivotal role of metabolomics for the synthetic microbiology of secondary metabolism, including its crucial role in novel compound discovery in microbes, the examination of side products of engineered metabolic pathways, as well as the identification of major bottlenecks for the overproduction of compounds of interest, especially in combination with metabolic modeling. We conclude by highlighting remaining challenges and recent technological advances that will drive metabolomics towards fulfilling its potential as a cornerstone technology of synthetic microbiology.

1. Introduction

Secondary metabolites constitute an important class of highly valuable compounds covering a broad spectrum of applications, including drugs (e.g. antibiotics, antitumor agents, immunosuppressants), agrochemicals (e.g. pesticides, insecticides, antifeedants), biofuels (e.g. squalene, oleoresin) and food additives (e.g. carotenoids, flavonoids, essential oils). A statistical estimate in 2005 reported approximately 23,000 known bioactive microbial metabolites, of which about 16,500 demonstrated antibiotic activities [1]. However, these compounds are usually produced in very low amounts (or not at all) under typical laboratory conditions in the species from which they originate. Fortunately, recent advances in synthetic microbiology may provide a potential alternative way to access this treasure trove of natural products.

Synthetic biology, which aims to redesign biological systems for novel purposes and applications, enables the transfer of a secondary metabolite biosynthetic pathway from its organism of origin into more amenable heterologous hosts, where the compounds of interest or their precursors can be produced with desired titers [2–7].

One important tool in the synthetic biology toolbox is metabolomics, which catalogues the entire complement of small metabolites in a biological sample [8–11]. General metabolomics applications in synthetic biology have been recently reviewed by Ellis and Goodacre [12], who focused on the integration of metabolomics, fluxomics and metabolic modeling in the design and optimization of engineered microbes.

In this review, we aim to illustrate the role of metabolomics specifically as a research tool in the synthetic biology of secondary metabolism. We first describe the importance of microbial secondary metabolism for synthetic biology. We then discuss the potential of exploiting metabolomics to discover novel compounds and biochemical pathways in microbes. The pivotal role of metabolomics in pathway engineering is further illustrated with examples on the identification of side products and major bottlenecks for the overproduction of compounds of interest. Furthermore, we discuss the potential of metabolomics, integrated with metabolic modeling, as the basis for large-scale synthetic biology projects: metabolomics can be used to supply key information for the improvement of predictive models and contribute to the computer-aided design of synthetic pathways. Finally, we highlight important breakthroughs in analytical methodologies that can support the most recent trends in synthetic microbiology.

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2. Secondary metabolism and synthetic biology

Bacterial genome mining has revealed numerous orphan secondary biosynthetic gene clusters, which potentially encode for novel compounds of high biotechnological value. These gene clusters constitute an almost inexhaustible natural resource of secondary metabolites for synthetic microbiologists [13–16]. The highly modular nature of the biosynthetic machinery responsible for the production of secondary metabolites makes them a particularly attractive target for synthetic biology strategies, both by refactoring gene clusters to produce their product more efficiently [17] and by recombining the modules to increase the fraction of chemical space accessible to biological production systems [18]. This rational engineering approach can further be enhanced by integration with random mutagenesis and metabolic modeling [2,8].

Recent advances in genome synthesis [9,10,19,20] make such strategies realistic on a relatively large scale. An outstanding example of engineering secondary metabolite biosynthesis is the overproduction of the artemisinin precursor artemisinic acid using a synthetic biology approach [21]. Genes encoding for the enzymes participating in consecutive steps in the artemisinin biosynthetic pathways were recruited from *Saccharomyces cerevisiae*, *Artemisia annua*, and *Escherichia coli*, assembled into two operons and transformed into an *E. coli* host strain; subsequently several optimization steps were performed in order to achieve efficient compound production [21,22]. Illustrating the power of a modular synthetic biology strategy, the same isoterpenoid pathway was also engineered towards the biosynthesis of taxadiene, a precursor for the clinically practiced anticancer drug taxol, achieving an increase in titer of approximately 15,000-fold in *E. coli* [23]. Currently, tools for similar biosynthetic engineering of typical secondary metabolite producers such as actinomycetes are also being developed [24].

The development of genome-reduced hosts for heterologous expression of engineered metabolic pathways of interest provides another important component to the synthetic biology toolbox, as it avoids interference from the complex endogenous secondary metabolome. One notable example illustrating the application of genome-minimized hosts for secondary metabolite production is the highly efficient expression of heterologous antibiotics (streptomycin, cephamycin C and pladienolide) and the plant isoterpenoid precursor, amorphadiene, in a genome-minimized strain of *Streptomyces avermitilis* [25].

3. Metabolomics and synthetic microbiology

Metabolomics is the comprehensive analysis of all (or, more realistically: many) metabolites in a biological sample. As metabolomics is the final step in the omics cascade, closest to the phenotype, it provides a direct snapshot of the physiological status of the cell at a certain time point and under specific circumstances [26]. Recent advances in metabolomics studies have been driven by breakthroughs in analytical methodologies in combination with software developments for interpreting experimental data [27]. Extensive research in the field of metabolomics is in turn a driving force for the improvement of the analytical instrumentation, especially in the case of mass spectrometry (MS) [28].

Mass spectrometry has long been a favorite platform for metabolomics studies thanks to its versatility in experimental design (global or targeted analysis, tandem MS for structural information), its high mass accuracy and its high sensitivity to identify and quantify (both relatively and absolutely) very low-abundance metabolites [29]. For microbiological applications, MS is most commonly used in combination with liquid chromatography (LC–MS), with gas chromatography (GC–MS) and capillary electrophoresis (EC–MS) used to a lesser extent [30,31]. All of these methods have been applied successfully for microbiological samples (reviewed in [32]), and the technology is now mature enough for large-scale applications [33]. In the synthetic biology of secondary metabolism, metabolomics can play important roles [34], both as a discovery and a debugging tool (summarized in Fig. 1).

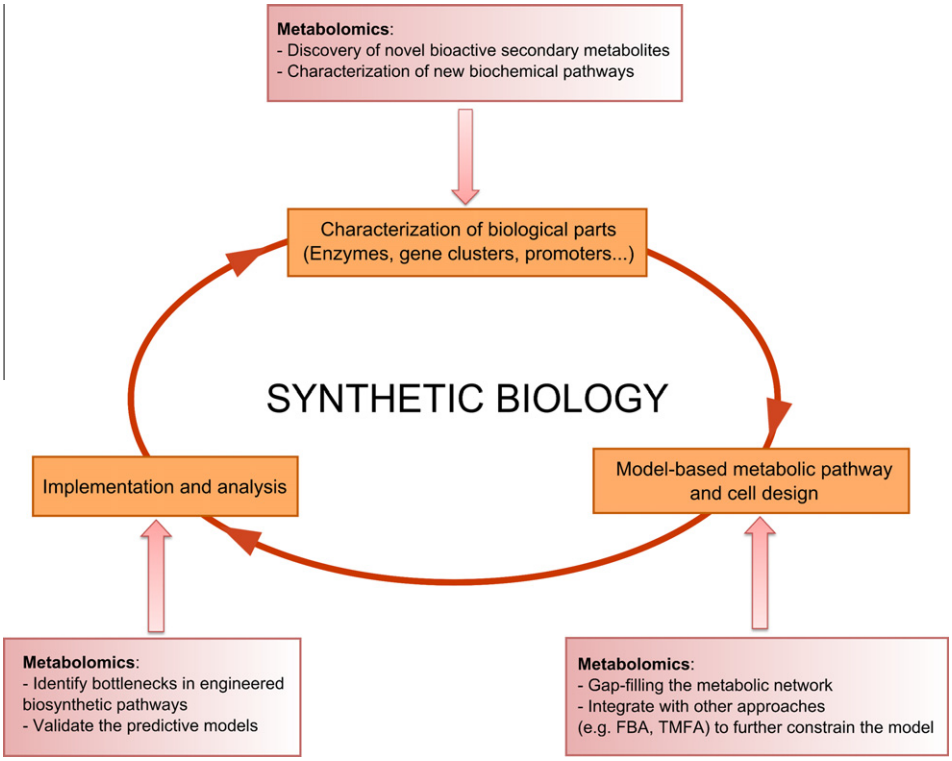

Fig. 1. The roles of metabolomics in the synthetic biology of secondary metabolism.