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Cell-free synthetic biology for *in vitro* biosynthesis of pharmaceutical natural products

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ABSTRACT

Natural products with significant biological activities continuously act as rich sources for drug discovery and development. To harness the potential of these valuable compounds, robust methods need to be developed for their rapid and sustainable production. Cell-free biosynthesis of pharmaceutical natural products by *in vitro* reconstruction of the entire biosynthetic pathways represents one such solution. In this review, we focus on *in vitro* biosynthesis of two important classes of natural products, polyketides (PKs) and nonribosomal peptides (NRPs). First, we summarize purified enzyme-based systems for the biosynthesis of PKs, NRPs, and PK/NRP hybrids. Then, we introduce the cell-free protein synthesis (CFPS)-based technology for natural product production. With that, we discuss challenges and opportunities of cell-free synthetic biology for *in vitro* biosynthesis of natural products.

Contents

1. Introduction

Nature has extraordinarily rich natural products, which are synthesized by living organisms ranging from tiny microorganisms to giant plants on this planet. Natural products are a large family of low molecular weight organic compounds with diverse chemical structures. These natural compounds have significant biological activities that act as abundant sources for drug discovery and development [1]. Over the past 30 years, more than 50% of new drugs available in the pharmaceutical market are natural products and their derivatives [2]. The important classes of natural products include, but not limited to, the well-known polyketides (PKs) and nonribosomal peptides (NRPs) that are produced by polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs), respectively, which are found in various microorganisms like the *Streptomyces* species [3]. PKs and NRPs possess a broad spectrum of biological activities (*e.g.*, antibiotic, immunosuppressant, and anti-cancer, *etc.*) and are used in many clinical applications [1,4]. For example, erythromycin (PK) and daptomycin (NRP) are clinically important antibiotics; the NRP/PK hybrid compound epothilone has been developed as an antitumor agent [1]. Due to their multiple uses in human medicine, the demand for these pharmaceutical natural products is continuously growing [2].

Traditionally, pharmaceutical natural products are extracted directly from their native producers like plants. However, these native producers often suffer from the low productivity of

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interesting molecules, demonstrating this solution is not environmentally friendly, sustainable, and cost-effective. Another strategy utilizes chemical synthesis to produce natural products of medicinal importance, however, the structural complexity of many natural compounds makes chemical synthesis hardly feasible or practical. As a current alternative approach, metabolic engineering and synthetic biology studies have tried to utilize surrogate microbes, for instance, *Escherichia coli* and *Saccharomyces cerevisiae*, to produce intricate pharmaceutical molecules by reconstitution of their entire gene clusters in the host cells [5–8]. Despite its success in the past, this approach still remains problematic to obtain high yields, which are mainly caused by metabolic burden inhibiting host cell growth, incorrect folding of heterologous proteins, lack of post-translational modification enzymes, and unavailability of necessary precursors in heterologous hosts. In order to tackle these *in vivo* problems, *in vitro*, cell-free, platforms have recently been developed and are emerging as powerful systems for the biomanufacturing of therapeutic proteins, low-value biocommodities, and value-added chemicals [9–13].

Generally, *in vitro* cell-free biomanufacturing systems separate cell growth (catalyst synthesis) from target product formation (catalyst utilization). Because of the absence of cell walls, these open cell-free systems allow for easy manipulation, monitoring, optimization, and sampling. In addition, *in vitro* cell-free platforms have many advantages over *in vivo* microbial systems, such as (i) high product yields that can be achieved by eliminating the synthesis/maintenance of cell biomass, removing undesired side pathways, and preventing the formation of by-products; (ii) fast reaction rates enabled by better mass transfer due to the lack of cell membrane; and (iii) tolerance of toxic precursors, intermediates, and products [9,10,14]. As a result, various products are produced via *in vitro* reconstruction of different biosynthetic pathways in a single reaction vessel. To this end, two cell-free systems are being commonly used: purified enzyme system and crude cell extract system [10,13].

Cell-free biosynthesis of proteins, bulk chemicals, and value-added compounds, *etc.* has been well summarized in several outstanding reviews [9,10,13,14]. In this review, we focus on cell-free biosynthesis of pharmaceutical natural products with an emphasis on PKs and NRPs (Fig. 1). First, we summarize purified enzyme-based natural product biosynthesis. Then, we introduce crude cell extract systems for natural product production, especially, with the cell-free protein synthesis (CFPS)-based technology. Finally, we discuss challenges and opportunities of cell-free synthetic biology for *in vitro* biosynthesis of natural products.

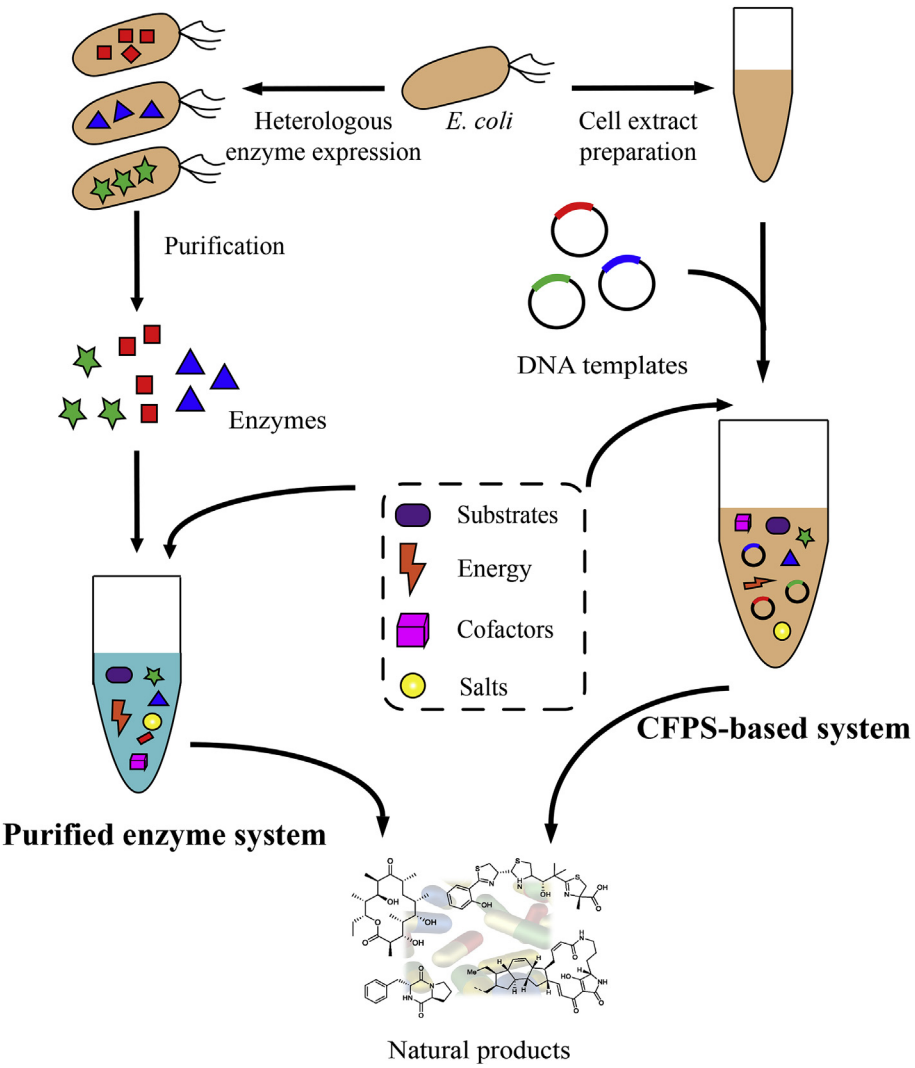

Fig. 1. *In vitro* biosynthesis of pharmaceutical natural products with purified enzyme-based system and cell-free protein synthesis (CFPS)-based system.

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