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Research review paper

Cell-free protein synthesis: Applications come of age

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ABSTRACT

Cell-free protein synthesis has emerged as a powerful technology platform to help satisfy the growing demand for simple and efficient protein production. While used for decades as a foundational research tool for understanding transcription and translation, recent advances have made possible cost-effective microscale to manufacturing scale synthesis of complex proteins. Protein yields exceed grams protein produced per liter reaction volume, batch reactions last for multiple hours, costs have been reduced orders of magnitude, and reaction scale has reached the 100-liter milestone. These advances have inspired new applications in the synthesis of protein libraries for functional genomics and structural biology, the production of personalized medicines, and the expression of virus-like particles, among others. In the coming years, cell-free protein synthesis promises new industrial processes where short protein production timelines are crucial as well as innovative approaches to a wide range of applications.

Contents

1. Introduction

Cell-free protein synthesis (CFPS) systems derived from crude cell extracts have been used for decades as a research tool in fundamental and applied biology (Fig. 1). They were used in the ground-breaking experiments of Nirenberg and Matthaei (1961), playing an essential role in the discovery of the genetic code. More recently, CFPS has shown remarkable utility as a protein synthesis technology (Katzen et al., 2005; Swartz, 2006), including the production of pharmaceutical proteins (Goerke and Swartz, 2008; Kanter et al., 2007; Yang et al., 2005; Zawada et al., 2011), and high-throughput production of protein libraries for protein evolution and structural genomics (Goshima et al., 2008; Griffiths and Tawfik, 2003).

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The driving force behind the development of this technology has been its potential to rapidly express bioactive recombinant DNA (rDNA) proteins. In particular, cell-free systems have distinct advantages over *in vivo* methods for rDNA protein production (Katzen et al., 2005; Swartz, 2006; Zawada et al., 2011). Without the need to support ancillary processes required for cell viability and growth, CFPS allows optimization of the cell extract towards the exclusive production of a single protein product. The absence of a cell wall enables an open and versatile environment for active monitoring, rapid sampling, and direct manipulation of the protein synthesis process. Finally, the cell-free format allows for screening without requiring a gene-cloning step (Fig. 2), enabling rapid process/product development pipelines (Kanter et al., 2007; Zawada et al., 2011).

Despite many promising aspects of cell-free systems, several obstacles have previously limited their use as a protein production technology. These obstacles have included short reaction durations of active protein synthesis, low protein production rates, and difficulty in supplying the intense energy and substrate needs of protein synthesis without deleterious concomitant changes in the chemical environment. Furthermore, expensive reagent costs (particularly high energy phosphate chemicals in the form of nucleotides and secondary energy sources), small reaction scales, a limited ability to correctly fold proteins containing multiple disulfide bonds, and its initial development as a "black-box" science were limitations (Swartz, 2006). However, technical advances in the last decade have addressed these limitations and revitalized CFPS systems to meet the increasing demands for protein synthesis (Katzen et al., 2005). Moreover, a recent demonstration of cost-effective cell-free protein synthesis in a 100-liter reaction by Sutro Biopharma, Inc. (Zawada et al., 2011) shows the potential of CFPS systems to become a powerful recombinant DNA protein production platform at the industrial scale.

In this review, we focus on developments that have transformed crude extract CFPS systems into a platform technology for industrial and high-throughput protein production. With due respect to the many advances in purified translation systems, such as the PURE system developed by Ueda and colleagues (Ohashi et al., 2010) as well as New England Biolabs (Asahara and Chong, 2010; Hillebrecht and Chong, 2008), we concentrate on crude extract based systems because the expense of the PURE system currently restricts large-scale commercial applicability. In addition, a review on the PURE system was recently published (Ohashi et al., 2010). Here, we begin with a brief introduction describing the technological capabilities of the field. In the next section, we discuss historical trends in protein yields, cost, reaction duration, and scale of CFPS systems. Finally, we examine frontier applications made possible by the recent technical renaissance.

2. Cell-free protein synthesis primer

To produce proteins of interest, CFPS systems harness an ensemble of catalytic components necessary for energy generation and protein synthesis from crude lysates of microbial, plant, or animal cells. Crude lysates contain the necessary elements for transcription, translation, protein folding, and energy metabolism (e.g., ribosomes, aminoacyl-tRNA synthetases, translation initiation and elongation factors, ribosome release factors, nucleotide recycling enzymes, metabolic enzymes, chaperones, foldases, etc.). Activated catalysts within the cell lysate act as a chemical factory to synthesize and fold desired protein products upon incubation with essential substrates, which include amino acids, nucleotides, DNA or mRNA template encoding the target protein, energy substrates, cofactors, and salts. After initiation of cell-free protein synthesis, production typically continues until one of the substrates (e.g., ATP, cysteine, etc.) is depleted or byproduct accumulation (e.g., inorganic phosphate) reaches an inhibitory concentration.

Although any organism can potentially provide a source of crude lysate, the most common cell-free translation systems consist of extracts from *Escherichia coli* (ECE), rabbit reticulocytes (RRL), wheat germ (WGE), and insect cells (ICE). Since these cells behave very differently, the extracts derived from them do as well. Thus, the first decision when attempting to produce biologically active proteins using CFPS is choosing the source of extract. Typically this decision begins by considering the availability of materials and convenience of extract preparation, yield of protein needed, protein origin and complexity, downstream processing needs, and cost. In the remainder of this section we highlight the most commonly used CFPS systems (Table 1).

The prokaryotic *E. coli* CFPS system is the most popular and is commercially available. The adoption of the *E. coli* system is due to several factors. First, *E. coli* is easily fermented in large quantities using low-cost media and easily ruptured using high-pressure homogenizers. Thus, extract preparation is simple and inexpensive. Second, *E. coli* based systems generally achieve the highest protein yields, from hundreds of micrograms per milliliter to milligrams per milliliter in a batch reaction, depending on the protein of interest (e.g., 1.7 mg mL^{-1} chloramphenicol acetyl transferase (Kim et al., 2011), 0.7 mg mL^{-1} human granulocyte-macrophage colony-stimulating factor (Zawada et al., 2011), and 0.022 mg mL^{-1} FeFe hydrogenase (Boyer et al., 2008)). Third, the reaction cost of the *E. coli* system is the lowest. This is due in large part to the ability to activate metabolic reactions in the extract that fuel high-level protein synthesis, which has obviated the need for using expensive energy substrates such as phosphoenolpyruvate (Swartz, 2006).

WGE, RRL, and ICE systems are the most widely used eukaryotic CFPS systems. They are also commercially available. Compared to the

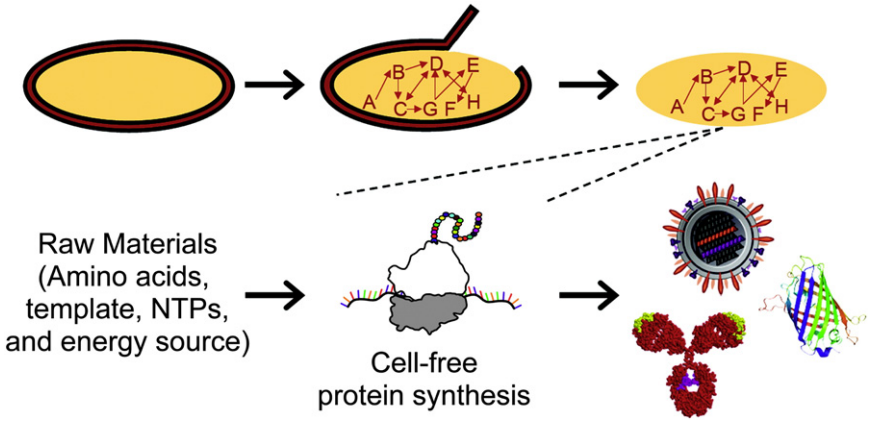

Fig. 1. Cell-free protein synthesis systems exploit crude cell extracts to produce valuable therapeutics and vaccines, among other products.