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Cell-free protein synthesis: Search for the happy middle

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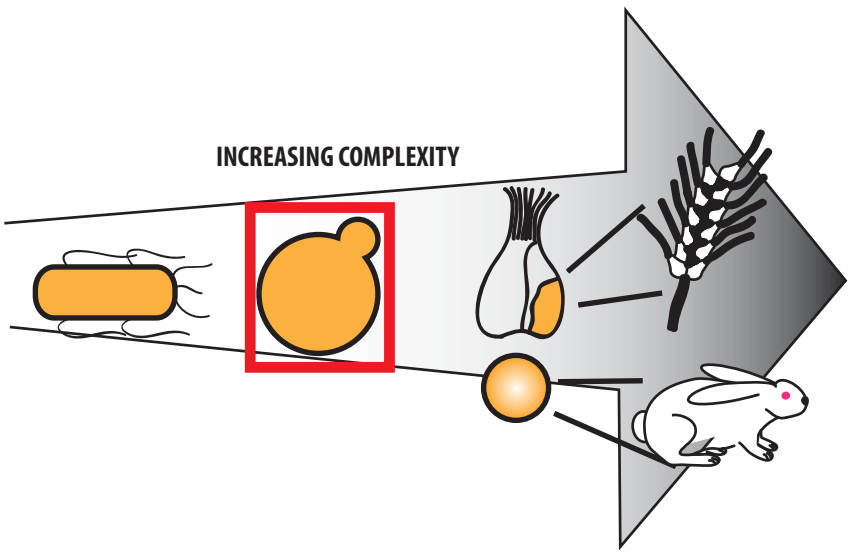
Cell-free protein synthesis (CFPS) is a versatile technique gaining popularity because it allows researchers access to on-demand production of proteins that integrate unnatural amino acids [1], radiolabeling [2], membrane insertion [3], and a whole range of post-translational modifications [4]. In the current issue of *Biotechnology Journal*, Gan and Jewett [5] outline a convenient and cost-effective methodology for the preparation of *Saccharomyces cerevisiae*-based CFPS reactions.



E. coli	*S. cerevisiae*	Wheat germ and rabbit reticulocyte
Pros • High protein productivity for prokaryotic and simple proteins • Great genetic tools for strain modification • Extensive systems biology characterization • Inexpensive large-scale fermentation	**Pros** • Possesses eukaryotic translational and folding machinery • Great genetic tools for strain modification • Extensive systems biology characterization • Inexpensive large-scale fermentation	**Pros** • Able to express multi-domain and complex proteins • More closely resemble native expression conditions for plant and animal proteins • Competent (with additives) for many post-translational modifications (glucosylation, prenylation, etc.)
Cons • Lacks chaperones and other machinery required for expressing and folding many eukaryotic proteins • Lacks eukaryotic post-translational modification systems	**Cons** • May lack appropriate chaperones for some proteins form higher eukaryotes (e.g., plants and animals) • Not commercially available	**Cons** • Limited genetic modification of cell-free extract source organism • Difficult and expensive to scale up

Figure 1. Pros and cons of cell-free protein synthesis systems: comparing *E. coli*, yeast, and wheat germ/rabbit reticulocyte

By eschewing the transformation, outgrowth, clone selection, induction, and lysis steps typically required for in vivo expression, CFPS permits high-throughput applications and a dramatically shortened turnaround time of hours instead of days, all while avoiding a number of host-protein interactions thus preventing formation of toxic proteins in vivo. However, CFPS does so at the cost of lower protein yields, more expensive reagents, and post-translational capabilities limited by those of the source organism from which the cell-free extract was prepared. Commercially available CFPS kits are largely prepared from *Escherichia coli*, wheat germ, or rabbit reticulocyte lysates. These extract sources each has respective tradeoffs: *E. coli* offers relatively high yields of protein without post-translational modifications, while wheat germ and rabbit reticulocyte lysate suffer from lower yields of protein but offer superior expression of complex, multi-domain proteins and/or more complicated modifications [6]. The yeast *S. cerevisiae* falls between these two extremes (Fig.1) but yeast CFPS kits are not yet commercially available.

In the article by Gan and Jewett [5], the authors optimized a number of conditions, including temperature, reaction time, and nucleotide concentrations, but their biggest contribution comes in finding an optimal capless translation-initiating untranslated region (UTR) to obviate the need for expensive 5′-capping of mRNA. As seen in other wheat germ CFPS [7] as well as in vivo in yeast [8], a short 5′ UTR sequence (Ω) from tobacco mosaic virus (TMV) proved to be an

excellent capless translation initiator, and the 3' untranslated regions (UTRs) tested were no better than a simple 50-nucleotide polyA tail [5]. The size of these flanking regions allowed the authors to develop a convenient, two-step PCR protocol to convert a gene into a linear template appropriate for CFPS within hours. From there, a 90-minute reaction generates up to 12.5 μg/mL of protein [5]. Thus, the process from cDNA to protein could take place within a workday.

As with *E. coli*, *S. cerevisiae* can be easily scaled to produce large amounts of cell biomass from which CFPS reactions can be derived. Additionally, these two organisms have ample genetic tools to allow even more control over the CFPS starting material. Future work may use genetic modifications to create a strain of *S. cerevisiae* optimized for cell-free work by including constitutively produced T7 RNA polymerase and creatine phosphokinase to remove the need to add these proteins separately, as well as knockout of housekeeping proteins such as XRN1 that degrade uncapped RNA [9]. Expressing accessory proteins, such as plant HSP101, may also help the TMV Ω load ribosomes to initiate translation [8]. A companion microsomal membrane or detergent additive system could be developed to make expression of transmembrane and ER-derived proteins more successful [3]. With the contributions of the current paper [5], yeast lysate is on its way to becoming the happy middle in CFPS, putting industrial-scale protein production and massively parallel high-throughput assays within reach in the same system, even for proteins requiring post-translational modifications and eukaryotic chaperones.

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