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Review

Wheat germ systems for cell-free protein expression

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ABSTRACT

Cell-free protein expression plays an important role in biochemical research. However, only recent developments led to new methods to rapidly synthesize preparative amounts of protein that make cell-free protein expression an attractive alternative to cell-based methods. In particular the wheat germ system provides the highest translation efficiency among eukaryotic cell-free protein expression approaches and has a very high success rate for the expression of soluble proteins of good quality. As an open *in vitro* method, the wheat germ system is a preferable choice for many applications in protein research including options for protein labeling and the expression of difficult-to-express proteins like membrane proteins and multiple protein complexes. Here I describe wheat germ cell-free protein expression systems and give examples how they have been used in genome-wide expression studies, preparation of labeled proteins for structural genomics and protein mass spectroscopy, automated protein synthesis, and screening of enzymatic activities. Future directions for the use of cell-free expression methods are discussed.

1. Introduction

Wheat germ extracts have been used for long in cell-free protein expression. Already in 1973, Roberts and Paterson described an S-30 (30 000×*g* supernatant) extract prepared from commercial wheat germ that efficiently translated RNA from tobacco mosaic virus and the rabbit globin 9S RNA [1]. They noticed the advantages of using an easily available starting material for rapid extract preparation that allowed for RNA dependent protein synthesis even of large polypeptides of over 50 000 Daltons. Following the initial work from Roberts and Paterson, others groups applied wheat germ extracts to the translation of more RNA templates, where factors like for instance energy supply, extract concentration, spermine, pH, Mg^{2+} and K^{+} concentration were found important for effective translation [2–4]. The wheat germ system could be further improved by continuous supply of amino acids and energy resources in combination with removal of inhibitory byproducts [5] largely increasing the potential of all cell-free protein synthesis methods in general [6]. While becoming widely used in biochemical research following the initial publications [7], however, in those early studies also limitations of the wheat germ extracts were noticed like the higher RNase activity in the extracts as compared to other systems [8], or the considerable variations in extract activity between different batches of wheat germ [3,4]. Although the original wheat germ extracts had only limited protein synthesis activity and were often instable, wheat germ extracts proved very valuable for protein research. Therefore a highly potent cell-free wheat germ protein synthesis system was developed in the last 15 years [9–11] that overcame all the limitations of the earlier methods and can rapidly produce milligram amounts of high quality protein. These wheat germ extracts with very high translation activity are today often used in many applications such as antigen production, determination of protein structures, or the development of functional assays. This eukaryotic expression system is suitable for the expression of both prokaryotic and eukaryotic proteins and does commonly not require codon optimization even for the expression of transcripts with a high A/T (e.g. Plasmodium) or G/C (e.g. Thermophiles) content. Hence, the wheat germ system is an attractive alternative to the many cell-based systems used in protein production. The successful use of wheat germ expression systems in many laboratories and large-scale projects contributed to the general progress made in cell-free protein synthesis and emphasizes that cell-free protein synthesis is today an established technology with important applications in research, applied science, and commercial processes [12–15]. Refer to Table 1 for an overall description of the wheat germ system and how it compares to other commonly used cell-free protein expression methods (*Escherichia coli* [16], *Leishmania tarentolae* [17,18], insect

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Table 1
Comparison of different cell-free protein expression systems.

System	Advantages	Disadvantages
E. coli [16]	• Established systems • Tested for many proteins • Low cost • Easy to make extracts • Very high translation speed • Very high protein yield • Genetic modification of strains possible • Recombinant system available (PURE)	• Many eukaryotic proteins insoluble • No post-translational modifications • Codon usage optimization is preferable
Leishmania tarentolae (LTE) [17,18]	• Eukaryotic system • Inexpensive • Better solubility of expressed proteins as compared to *E. coli* system • Can produce ng to mg amounts of protein	• New system • Tested for limited number of proteins • Protein modification not studied in this system • Few references in the literature
Wheat germ	• Well established system • Tested for many proteins • Best yield for eukaryotic system • Very high solubility rate • Synthesis of very large proteins demonstrated (~200 kDa range) • Cap independent translation • Commonly no codon optization needed • Stable system allowing work with different additives	• Extract preparation time consuming • Some background phosphorylation was observed in protein MS studies • No glycosylation
Insect [19]	• Translation of large proteins • Cap independent translation • N-glycosylation possible • Formation of disulfide bridges • Used in combination with vesicles	• New system • Lower protein yields than *E. coli* or wheat germ systems • Higher cost • Tested for few proteins
Rabbit reticulocyte [20]	• Old but very well established system • Tested for many proteins • Mammalian system • Cap independent translation • Often used in research	• Treatment of animals required • Sensitive to additives • No glycosylation • Co-expression of off-target proteins • Hemoglobin concentration ~90% of protein
Human [21]	• Some protein modification	• Low yield • Sensitive to additives • Tested for limited number of proteins

cells [19], rabbit reticulocytes [20], and human [21]) that will not be further described in this review.

2. The wheat germ system

The Endo group at Ehime University in Japan was the first to investigate reasons for the instability of wheat germ extracts. Based on their observation that RNA *N*-glycosidase tritin and other inhibitors like thionin, ribonucleases, deoxyribonucleases, and proteases are mostly derived from the endosperm, they developed a protocol for the removal of endosperm contaminations from wheat germs [9]. Extensive washing of wheat embryos yielded in stable extracts having a very high translation activity. Applying such wheat germ extracts, a general cell-free protein expression system was first established for high-throughput proteomics [10,22]. This system further provided for an optimal expression vector (pEU, [23]) and a special primer set for direct template preparation by PCR (Fig. 1). Using the natural omega (Ω) translational enhancer from tobacco mosaic virus, RNA transcripts could be prepared that did not require 5′- capping and a poly(A) tail for effective translation. It was rather found that the translation efficiency of a transcript was mostly dependent on the length of the 3′-untranslated region (~1500 nucleotides are recommended) protecting the RNA against degradation by 3′ to 5′ exonucleases. For increasing the throughput of the system, expression templates having a SP6 RNA polymerase promoter can be directly prepared by a special PCR method [10]. This method avoids unspecific expression from primer artefacts or primer dimers, because the SP6 promoter sequences are not part of a single primer, but are generated from two primers in two consecutive PCR steps ("Split Primer PCR"). By repeated supply of an mRNA template, protein translation in this system could be maintained for 14 days to produce some 9.7 mg of Green Fluorescent Protein (GFP) in 1 ml reaction volume demonstrating the high stability of the translation machinery in those wheat germ extracts [10]. Efficient cloning systems for template construction for the wheat germ system have been published [24–26], and additional expression vectors for the wheat germ system beyond pEU are available in the public domain [27]. An improved omega (Ω) translational enhancer has also been described for the wheat germ system, which better controls translational initiation [28], and there are procedures for achieving higher yields working with pEU-originated templates on a high-throughput [29]. Optimal translational enhancers for the wheat germ system can be further selected using random sequences and bio-evolutionary techniques [30,31]. Such an approach was used to prepare the artificial enhancer in vector pEU01 [32], which is frequently used for expressing proteins in the wheat germ system.

Cell-free protein expression can be performed in so-called "coupled" systems running RNA transcription and protein translation at the same time in the same reaction mixture. The wheat germ cell-free protein expression system, however, often uses two "linked" reactions, where in the first step the RNA is prepared from a circular or linear DNA template using preferably the SP6 RNA polymerase. As an alternative T7 RNA polymerase may be used as well [9], although it can give lower yields than the SP6 RNA polymerase when working with circular DNA templates. In the second step, the RNA from the first reaction is then utilized for protein translation (Fig. 1). Even though the linked reactions require more setup and reaction time, only the separate reactions allow to do transcription and translation under optimal reaction conditions, to easily setup complex translation reactions with multiple RNA templates [33], and to use additives in the translation reaction without interfering with transcription. It had been shown, for

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