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Optimizing antibody expression: The nuts and bolts

B. Vijayalakshmi Ayyar [a], Sushrut Arora [b,*], Shiva Shankar Ravi [a]

[a] Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA
[b] Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA

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ABSTRACT

Antibodies are extensively utilized entities in biomedical research, and in the development of diagnostics and therapeutics. Many of these applications require high amounts of antibodies. However, meeting this ever-increasing demand of antibodies in the global market is one of the outstanding challenges. The need to maintain a balance between demand and supply of antibodies has led the researchers to discover better means and methods for optimizing their expression. These strategies aim to increase the volumetric productivity of the antibodies along with the reduction of associated manufacturing costs. Recent years have witnessed major advances in recombinant protein technology, owing to the introduction of novel cloning strategies, gene manipulation techniques, and an array of cell and vector engineering techniques, together with the progress in fermentation technologies. These innovations were also highly beneficial for antibody expression.

Antibody expression depends upon the complex interplay of multiple factors that may require fine tuning at diverse levels to achieve maximum yields. However, each antibody is unique and requires individual consideration and customization for optimizing the associated expression parameters. This review provides a comprehensive overview of several state-of-the-art approaches, such as host selection, strain engineering, codon optimization, gene optimization, vector modification and process optimization that are deemed suitable for enhancing antibody expression.

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1. Introduction

The advent of monoclonal antibodies (mAbs) using hybridoma technology was a significant and a greatly successful breakthrough in biological research, providing scientists with a way to generate antibodies with singular specificity. Advances in genetic engineering took antibody generation technologies way further by making it possible to generate novel antibody modalities that even surpass Mother Nature's design, providing a completely new dimension to the antibody applications [1,2]. Recombinant antibodies (rAbs) overcame many bottlenecks associated with mAb production and

* Corresponding author.
E-mail address: drsarora@gmail.com (S. Arora).

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are currently one of the fastest growing sectors in the biopharmaceutical industry [3,4]. The nascent shift from monoclonal antibodies to recombinant antibodies was fueled by the development of powerful genetic engineering techniques providing the ability to manipulate antibody genes, making it feasible to generate antibodies with desired configuration, binding and effector properties, allowing facile incorporation of tags and fusion proteins to assist in detection, conjugation, purification, and increasing their serum half-lives [3–8]. Availability of high throughput screening platforms, ease of genetic material preservation, stable recovery and reproducibility of antibodies over time, ease of production in inexpensive microbial hosts along with the patenting aspect adds to their commercial value [3–7].

Expression is multistep process initiated by transcription of genetic information from deoxyribonucleic acid (DNA) to messenger RNA (mRNA) and culminating in translation of mRNA to protein (Fig. 1). Antibodies in general are complex structures, requiring sophisticated cellular machinery for proper expression, folding and processing. The demand for structural variability to suit the ever-demanding biomedical needs has yielded sophisticated antibody architectures (Fig. 2) exhibiting mono, di, tri and tetravalent binding abilities with molecular weights ranging from 12 to 150 kDa [9]. Expression is influenced by the size, sequence and format of antibodies, and the number of inter- and intra-disulfide bonds, and posttranslational modifications required for the overall functioning of the antibody. Optimal functional expression and purification of these superior antibody designs are crucial factors in deciding their fate for practical applications. For example, bispecific antibodies suffered a long period of dormancy owing to problems with low expression yields and product instability [3,10]. Not until recently, bispecific antibodies were revived again using modern molecular biology tools that helped circumvent the challenges associated with their expression [3,10]. Majority of the rAbs are based on the antigen binding unit of the immunoglobulin structure, which is sensitive to the conformational changes and may lead to aggregation of the expressed antibodies rendering it unsuitable for application [11]. In the absence of a universal platform guaranteeing good functional yield of rAbs, it is imperative to carefully optimize the expression and purification conditions for each antibody individually (Fig. 3) in order to achieve maximum outputs. This review aims to summarize different strategies and recent trends, explored for optimizing antibody expression.

2. Selection of expression hosts

Selection of an appropriate host is the first step in obtaining optimum antibody expression. An array of prokaryotic or eukaryotic hosts are available for antibody expression possessing their own set of advantages and disadvantages (Table 1) (reviewed by [4,12]). Mammalian cell lines (most commonly CHO or HEK293) are preferred for expression of therapeutic antibodies due to their low immunogenicity, and in-built cellular machinery responsible for optimum folding, secretion and posttranslational modifications. However, mammalian hosts have certain drawbacks, such as smaller library sizes, expensive production costs and difficulties in genetic manipulation, making microbial hosts the most convenient choice for rAb production.

Among microbial expression hosts, *Escherichia coli* undoubtedly remains the most exploited expression system in research settings [4,13]. Its popularity is due to the ease of production and handling, inexpensive culture methods, availability of an array of

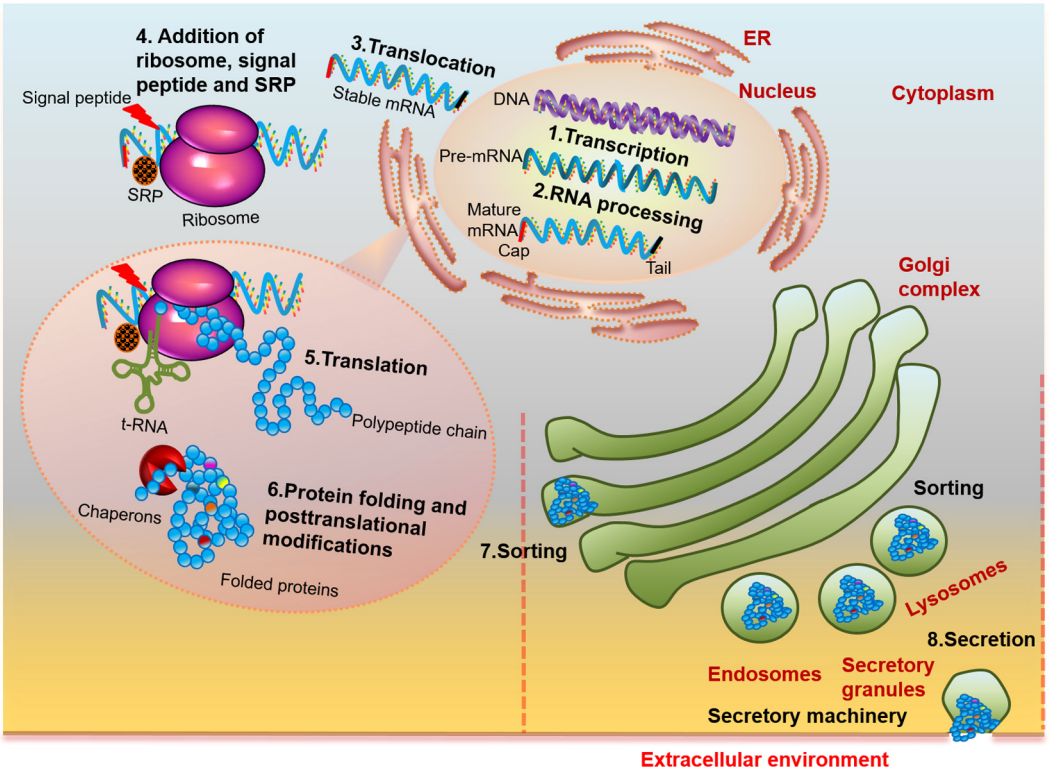

Fig. 1. An overview of the cell machinery required for protein synthesis and its secretion. The figure shows a schematic of an eukaryotic cell with some of its membrane bound organelles (nucleus, endoplasmic reticulum, ribosomes and golgi complex). Genetic information is transcribed from DNA to mRNA in nucleus. Precursor mRNA is processed by addition of a cap at 5′ end, cleavage and polyadenylation at 3′ end, followed by splicing to remove non-coding sequences from the precursor mRNA, resulting in mature mRNA, which is transported to the cytoplasm. In cytoplasm, mRNA binds to ribosome, signal peptide and signal recognition particle (SRP) and with the help of tRNA gets translated into protein. Newly synthesized proteins undergo posttranslational modifications and are provided with signal sequences that direct/sort the proteins to their correct place. After sorting, the proteins destined to be secreted extracellularly, pass through golgi complex and are contained within secretory vesicles which fuse with the plasma membrane allowing the protein to be secreted.