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Debottlenecking protein secretion and reducing protein aggregation in the cellular host

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Chinese hamster ovary (CHO) cells have been extensively used for industrial production of biotherapeutics. With advances in cell line development and process optimization, production levels of therapeutic proteins using the CHO expression system have increased to beyond 10 g per liter scale. These high-titer processes could challenge the secretory capacity of CHO cells, which can result in degradation and aggregation of the protein of interest. This review discusses bottlenecks in the secretory pathway of CHO cells that lead to inefficient secretion and aggregation of proteins, and summarizes current strategies to tackle these bottlenecks. In addition, emerging technologies that facilitate better understanding of cellular mechanisms in protein production could provide new avenues to improve the secretion and quality of protein therapeutics.

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Introduction

Chinese hamster ovary (CHO) cell lines have been used extensively to produce recombinant therapeutics. In the past two decades production levels of therapeutic proteins using the CHO cell system have increased remarkably [1]. Optimized cell line screening technologies, chemically defined media formulations and novel process analytical technologies have enabled processes with high volumetric productivity to meet commercial demand [2]. Cell culture process titers of at least 5 g/L are the current industry standard for monoclonal antibodies with up to 13 g/L being reported in fed batch production [3]. The capability of the cellular protein folding and secretion machinery to handle these high-titer processes becomes important in context of setting the baseline for desirable product quality. Moreover, high concentrations of heterologous proteins in CHO cells can go beyond the capacity of CHO cell secretion machinery, leading to inefficient secretion, protein degradation, intracellular and extracellular aggregation [4]. This review will focus on cellular mechanisms that could lead to inefficient secretion and aggregation of the protein therapeutics and will discuss mitigation strategies.

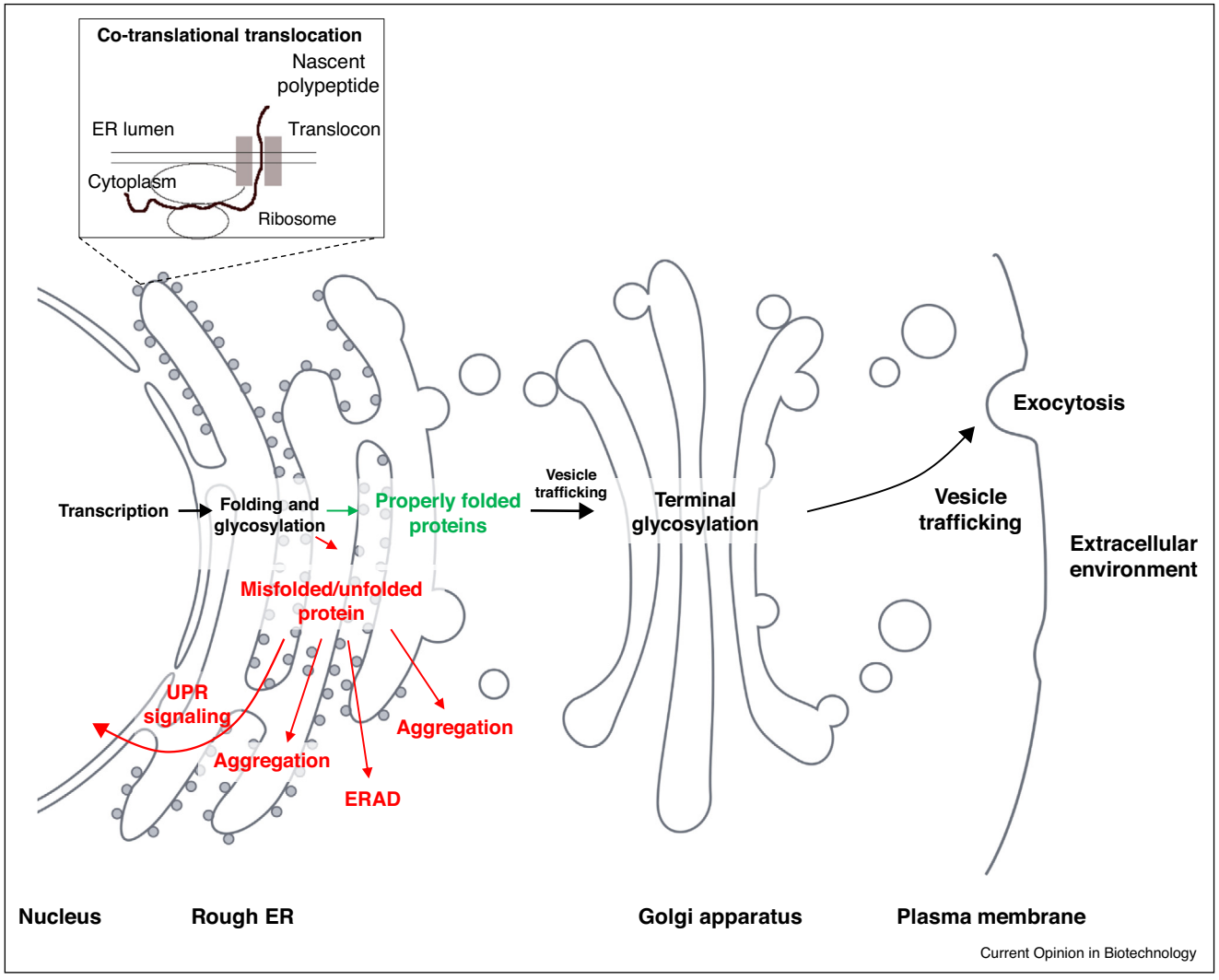
Bottlenecks in cellular and cell culture processes that lead to inefficient secretion and aggregation of therapeutic proteins

Protein secretion in mammalian cells starts from translocation of the polypeptide into endoplasmic reticulum (ER), followed by protein folding and maturation in the ER lumen, trafficking to Golgi for additional glycosylation and sorting, and finally secretion into the extracellular environment (Figure 1). Stringent quality control machinery including unfolded protein response (UPR) and ER-associated-protein-degradation (ERAD) regulates protein homeostasis in CHO cells [5]. However, the quality control machinery in CHO cells may be overwhelmed due to the high transcription and/or translation levels of target molecules. Therapeutic proteins additionally may be large complexes which are intrinsically more challenging to fold properly compared to endogenous proteins [4,6]. Potential bottlenecks in the secretion pathway include inefficient translocation of the nascent polypeptide chain into ER, mis-cleavage of the signal peptide, improper folding which leads to degradation and/or intracellular aggregation, ER stress and stress-induced apoptosis, and inefficient vesicular trafficking [4].

Bottlenecks in the secretion pathway, along with extended exposure to cell culture conditions, can further result in intracellular and extracellular aggregation of the protein therapeutics. Aggregation arises from different mechanisms based on protein characteristics or protein–protein interactions resulting in soluble/insoluble, covalent/non-covalent or reversible/irreversible aggregates [7]. While aggregates can typically be minimized through removal in downstream processing, they may result in a reduction of recoverable protein yields or may seed additional aggregates, thus affecting stability and limiting product shelf life.

Multiple studies have reported bottlenecks that affect the secretion and aggregation of protein therapeutics. For example, Le Fourn *et al.* identified that improper

Figure 1



The protein secretion and folding pathway in mammalian cells. Proteins of interest are translated on the rough ER membrane by ribosomes. The newly synthesized polypeptides are simultaneously translocated into the ER-lumen. Folding and maturation of the proteins are assisted with ER-chaperone proteins and foldases. Accumulation of unfolded or misfolded proteins triggers the unfolded protein response (UPR) which aims to restore protein homeostasis in ER. Folding-incompetent proteins are removed by ER-associated-degradation (ERAD) in the proteasome, whereas properly folded proteins travel to Golgi apparatus in ER-budded membrane vesicles. Vesicle fusion to the target membrane is mediated by SNARE proteins. Additional post-translational modification such as glycan extension occurs in Golgi. Secretory proteins are then sorted to the plasma membrane and secreted via exocytosis.

functioning of the signal recognition particle (SRP) complex during polypeptide translocation into ER resulted in mis-cleavage of the signal peptide, light chain aggregation in ER, and low yields [8]. Intracellular aggregation of heavy chain was also reported, possibly due to inefficient assembly with light chain [9]. In addition, intracellular accumulation of heterologous proteins and elevated levels of ER stress-induced proteins imply insufficient folding and secretion capacity [10,11,12•]. Furthermore, a suboptimal extracellular environment can induce protein aggregation upon secretion. Reducing agents like cysteine can be oxidized by metals in the medium formulations to drive Fenton reactions generating radicals that modify the protein and cause extracellular aggregation [13••]. While downstream processes and formulation conditions are streamlined to reduce aggregates, cellular engineering and cell culture process optimization provide opportunities to minimize aggregation upfront and simplify downstream operations.

Strategies to tackle bottlenecks in the secretion pathway and mitigate aggregation

Genetic engineering

Most attempts to resolve bottlenecks in the secretion pathway seek to engineer expression vectors or cellular