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Review Article

Enhancing recombinant antibody yield in Chinese hamster ovary cells

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Abstract

A range of recombinant monoclonal antibodies (rMAbs) have found application in treating diverse diseases, spanning various cancers and immune system disorders. Chinese hamster ovary (CHO) cells have emerged as the predominant choice for producing these rMAbs due to their robustness, ease of transfection, and capacity for posttranslational modifications akin to those in human cells. Transient transfection and/or stable expression could be conducted to express rMAbs in CHO cells. To bolster the yield of rMAbs in CHO cells, a multitude of approaches have been developed, encompassing vector optimization, medium formulation, cultivation parameters, and cell engineering. This review succinctly outlines these methodologies when also addressing challenges encountered in the production process, such as issues with aggregation and fucosylation.

Keywords: *Cell engineering, Chinese hamster ovary cells, Plasmid construction, Recombinant antibody*

Introduction

As members of the immunoglobulin (Ig) superfamily, antibodies (Abs) are glycoproteins secreted by plasma cells. Structurally, Abs consist of two heavy chains (HCs) and two light chains (LC) intertwined through both covalent and noncovalent interactions, forming three segments linked by a flexible hinge region. These segments include two antigen-specific binding (Fab) domains for attaching to antigens and one crystallizable fragment (Fc) domain responsible for initiating the effector functions. In human, based on the characteristics of the diverse constant region structures and properties of HC, Abs are divided into five classes: IgG, IgM, IgA, IgE, and IgD. Among these, IgG stands as the most prevalent class in serum, with a longer half-life, comprising 75% of circulating antibodies [1].

The pharmaceutical industry has thrived due to strong market demand, with global prescription drug sales surpassing $1 trillion USD in 2017 [2]. A significant proportion of biopharmaceuticals approved by the Food and Drug Administration of the USA consisted of recombinant monoclonal antibodies (rMAbs) or their conjugates. The global monoclonal antibody (mAb) market exceeded $100 billion USD in 2017 [2]. Since 2016, approximately 70% of all recombinant antibodies have been produced from Chinese hamster ovary (CHO) cell lines [3]. These rMAbs found application in treating various illnesses, encompassing cancers, autoimmune conditions, and inflammatory disorders [4-6]. With a projected market value set to exceed $300 billion by 2025, rMAbs stand at the forefront of biopharmaceuticals, leading in terms of therapeutic approvals [6]. In addition, truncated versions of monoclonal antibodies, such as Fab fragments, are employed in therapeutic and research contexts. The generation of rMAbs and (Fab) s employs a variety of expression systems. *Escherichia coli* is favored for producing Fab molecules due to its streamlined process, yielding gram/liter quantities within shorter timeframes. Various expression systems, aside from bacteria such as *E. coli*, have been employed for producing recombinant antibodies. A comparison of these expression systems is detailed in Table 1 [7,8]. For therapeutic purposes, rMAbs necessitate precise glycosylation and other posttranslational modifications to carry out their intended biological functions. Consequently, rMAbs approved for human therapy are limited to production in mammalian cells, such as CHO, mouse myeloma cell line (NS0), and Sp2/0 cells, attributed to their capability for intricate posttranslational modifications [9]. CHO cells are especially favored to produce rMAbs, owing to the likeness of the rMAbs generated in CHO cells to natural mAbs in terms of structure and function, resistance to human viruses, stable integration of exogenous genes, minimal secretion of endogenous proteins for ease of isolation, and suitability

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Table 1: Comparisons of recombinant antibody production across various expression systems (Table modified from Fisher and Emans [7], Goldstein and Thomas [8])

	Expression system				
	Bacteria	**Yeast**	**Insect cells**	**Mammalian cells**	**Plant cells**
Production					
Production time	Short	Short	Long	Long	Long
Product yield	Medium	High	Medium to high	Medium to high	High
Cost of cultivation	Cheap	Cheap	Expensive	Expensive	Expensive
Scale-up cost	High	High	High	High	Cheap
Production vehicle	Yes	Yes	Yes	Yes	Yes
Sensitive to shear stress	Medium	Medium	High	High	Unknown
Molecular perspective					
Glycosylation	Absent	Incorrect	Correct, strain dependent	Correct	Correct
Protein folding accuracy	Low	Medium	High	High	High?
Gene size	Unknown	Unknown	Limited	Limited	Unlimited
Risk					
Safety	Low	Unknown	Medium	Medium	Low
Therapeutic risk	High	Medium	Medium	High	Unknown
Public perception of risk	Low	Medium	Medium	Yes	Low

for large-scale suspension cultures [10]. Therapeutic rMAbs produced in CHO cells before 2020 have been documented in a previous review article [11]. Therefore, the therapeutic recombinant antibodies recently approved by the United States or European Union and produced in CHO cells are listed in Table 2. Remarkably, only one of the therapeutic recombinant antibodies approved by the United States or European Union in 2021 [12] and 20223 [13] was produced in NS0 cells, while the rest were manufactured in CHO cells. Hence, after years of dedicated research and development, CHO cells have emerged as the most prevalent host cell line for antibody production, primarily due to their capability to execute the necessary posttranslational modifications of monoclonal antibodies crucial for therapeutic efficacy.

In light of these advantages, therapeutic rMAbs are predominantly manufactured within CHO cells and subsequently subjected to chromatographic purification. The expression titer of the first recombinant therapeutic protein (tissue plasminogen activator) approved for marketing in 1986 was <50 mg/L [14]. After 30 years, the processes involved in CHO-based rMAb production have reached a high level of refinement, typically achieving peak product titers of around 1 g/L in batch processes and 1–10 g/L in fed-batch operations [15,16]. To augment rMAb yields in CHO cells, a range of strategies have been employed, including customized vector design, optimized growth medium formulations, controlled culture conditions, and potential cell engineering enhancements [10].

Construction strategies

The expression vector plays a pivotal role in driving the expression of heterologous rMAbs within CHO cells [11,17]. This vector essentially governs the degree and quality of rMAb expression in both transient and stable expression [18]. The steps involved in the expression of these plasmids, as illustrated in Figure 1, include transcription from the promoters, RNA splicing to generate mRNA, translation of mRNA, regulation of translation, and so forth. To enhance the efficiency of these exogenously expressed genes, various approaches have been employed in vector construction.

1. Promoter: Currently, the prominent promoters employed in CHO cell expression vectors are derived from the simian vacuolating virus 40 (SV40), human cytomegalovirus (CMV) immediate early (IE) promoter, and elongation factor-1α (EF-1α). Among these, the EF-1α promoter has the highest potency [19]. Modifications to promoters have demonstrated the ability to enhance antibody production in CHO cells[20]
2. 5′-Untranslated region (5’-UTR): An element that enhances translation has been discovered within the UTR of Hsp70 mRNA, increasing the translation efficiency of the cap-dependent structure [21]. The RNA structures within the 5′-UTR are acknowledged for their role in influencing translation efficiency. This insight offers a systematic approach to finely adjusting protein expression levels in mammalian cells, ultimately contributing to the improvement of recombinant protein expression [22]
3. Intron: In the modulation of foreign gene expression, introns have the capacity to enhance it. A comparative analysis of the impact of five distinct introns on transgene expression in CHO cells has been conducted previously. Among these introns, the SV40 intron demonstrated the highest level of transgene expression under both transient and stable transfection conditions. Notably, this intron also achieved a substantial elevation in recombinant protein production within CHO cells [23]
4. Signal peptide: The translocation of a newly synthesized protein from the cytosol to the endoplasmic reticulum (ER), facilitated by its signal peptide, stands as a pivotal stage in protein secretion, notably in the case of antibodies. The efficient production of the CYTL1 protein has been successfully achieved in the CHO cell expression system through the utilization of the mouse IgGκ signal peptide [24]. In addition, the optimization of signal peptides for both HC and LC has been demonstrated,

Table 2: List of therapeutic recombinant antibodies produced in Chinese hamster ovary cells recently approved by the United States or European Union

Trade name	Ingredient (s)	Target(s)	Developer(s)	Year of first approved
Evkeeza	Evinacumab	ANGPTL3	Regeneron pharmaceuticals	2021
Jemperli	Dostarlimab	PD-1	GlaxoSmithKline	2021
Rybrevant	Amivantamab	EGFR and MET	Janssen Biotech	2021
Aduhelm	Aducanumab	Beta-amyloid	Biogen Inc.	2021
Saphnelo	Anifrolumab	IFNAR1	AstraZeneca	2021
Bimzelx	Bimekizumab	IL-17A and IL-17F	UCB	2021
Tivdak	Tisotumab vedotin	CD142	Seagen Inc.; Genmab A/S	2021
Regkirona	Regdanvimab	SARS-CoV 2	Celltrion Inc.	2021
REGEN-COV	Casirivimab and imdevimab	SARS-CoV 2	Tarrytown	2021
Kimmtrak	Tebentafusp	HLA-A*02:01 complexed with a peptide from the melanoma-associated antigen gp100	Immunocore holdings plc	2022
Vabysmo	Faricimab	Ang-2 and VEGF-A	Roche; Genentech	2022
Enjaymo	Sutimlimab	Complement protein component 1	Sanofi	2022
Opdualag	Relatlimab	LAG-3	Selleck	2022
Evusheld	Tixagevimab/cilgavimab	SARS-CoV-2	AstraZeneca	2022
Lunsumio	Mosunetuzumab	CD20/CD3	Roche	2022
Tecvayli	Teclistamab	CD3; BCMA	Janssen research and development	2022
Spevigo	Spesolimab	IL-36	Boehringer ingelheim	2022
Imjudo	Tremelimumab	CTLA-4	AstraZeneca	2022
Beyfortus	Nirsevimab	RSV F protein	AstraZeneca; Sanofi	2022
Elahere™	Mirvetuximab soravtansine	Folate receptor α	ImmunoGen	2022
Tzield	Teplizumab	CD3	Provention Bio	2022

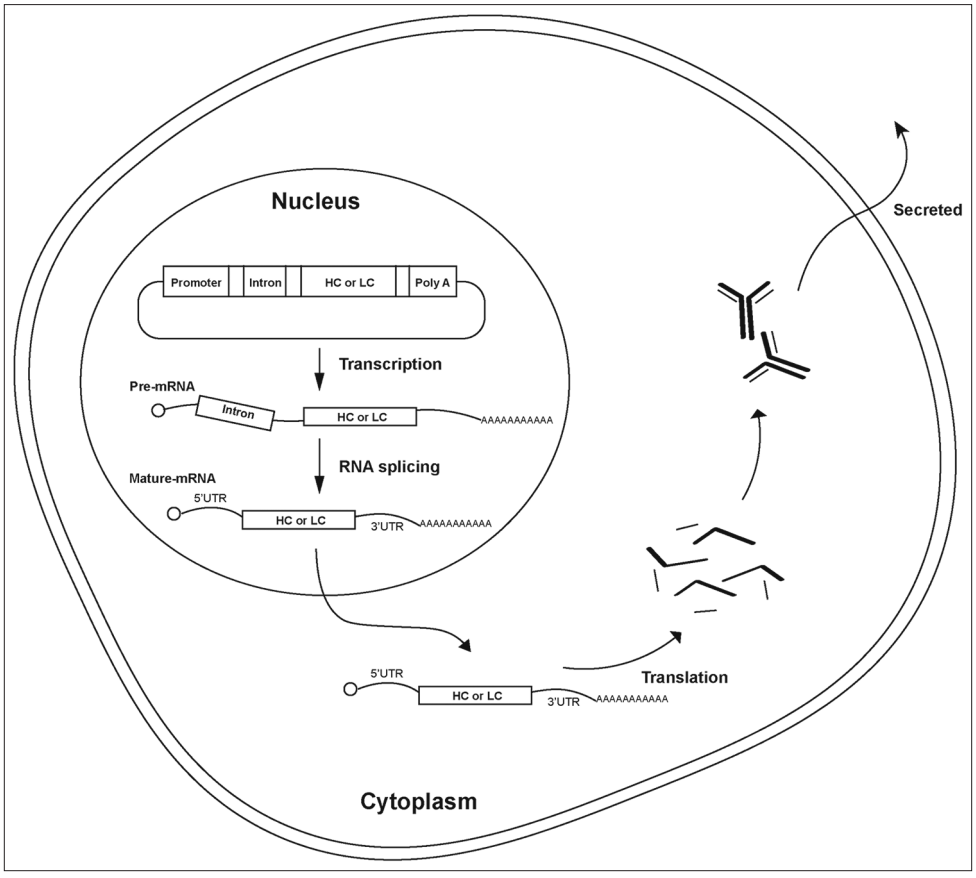

Figure 1: Expression of recombinant antibodies from expression plasmids in mammalian cells (e.g. Chinese hamster ovary cells). Several approaches have been utilized to enhance the recombinant antibody expression, such as promoter selection, incorporation of intron with 5'-UTR, codon optimization of expression genes, control the ratio of LC/HC, etc., HC: Heavy chain, LC: light chain, UTR: Un-translated region

showcasing their role in achieving high-efficiency expression of therapeutic antibodies in CHO cells [25]

5. Codon optimization: The substitution of the codon in the variable region of an antibody with the favored codon from the natural human antibody gene results in a notable enhancement of antibody expression levels in mammalian cells, exhibiting a substantial increase ranging from two- to threefold [26].

Refining the sequences within the codon and/or 5'-UTR should further boost the translation of HC and/or LC proteins, just like the optimization of mRNA UTR for improved expression of therapeutic mRNA and mRNA vaccines [27-30]. Indeed, gene optimization of the HC gene has been demonstrated to enhance its mRNA stability, and, in turn, to increase the antibody production [11,31].

In addition to the above-mentioned approaches to construct the expression plasmids, diverse strategies for vector design have emerged to facilitate rMAbs expression in CHO cells [32,33]. These strategies encompass monocistronic vectors, dual-promoter expression vectors, and bicistronic vectors mediated by elements such as internal ribosome entry sites (IRES) or Furin-2A [Figure 2].

A monocistronic vector expresses a single protein from the plasmid. To generate rMAbs within CHO cells, two separate monocistronic vectors are employed to express the HC and LC individually [Figure 2(I)].

In a dual-promoter expression vector, two distinct promoters regulate the transcription of HC and LC mRNAs, respectively [Figure 2(II)]. However, the capacity of a dual-promoter vector is inherently restricted, which poses challenges in accommodating more than two transcription units. This constraint curtails the application of this approach.

Bicistronic vectors, on the other hand, enable the joint expression of LC and HC in a single mRNA, driven by a single promoter. These vectors offer balanced production levels for both LC and HC proteins, resulting in heightened rMAb expression with minimal aggregation and consistent glycosylation. Two variations of bicistronic vectors can be formulated using IRES or Furin-2A peptide [Figure 2(III)]. IRES is a segment of noncoding sequences found within certain viral genomes, typically in the 5′-UTR of an RNA virus (e.g. encephalomyocarditis virus and hepatitis C virus). IRES facilitates cap-independent translation initiation, enabling the expression of multiple proteins from a singular mRNA transcript. On the other hand, the Furin-2A-mediated vector presents a viable option due to its efficient "self-cleaving" mechanism, ensuring the comparable expression of light and HCs from a single open reading frame [34]. The 2A peptide, comprising around 20 amino acids, acts as a "self-cleaving" peptide present in picornaviruses. Four types of 2A peptides are known, originating from foot-and-mouth (F2A), equine rhinitis (E2A), porcine teschovirus-1 (P2A), and Thosea asigna virus (T2A). The integration of the 2A peptide between LC and HC polypeptides triggers the production of distinct LC and HC segments through cotranslational cleavage at the C-terminus of the 2A polypeptide, resulting in balanced coexpression of HC and LC proteins [35].

The monoclonal antibody's expression level, facilitated by the F2A-mediated bicistronic vector, surpassed that of the

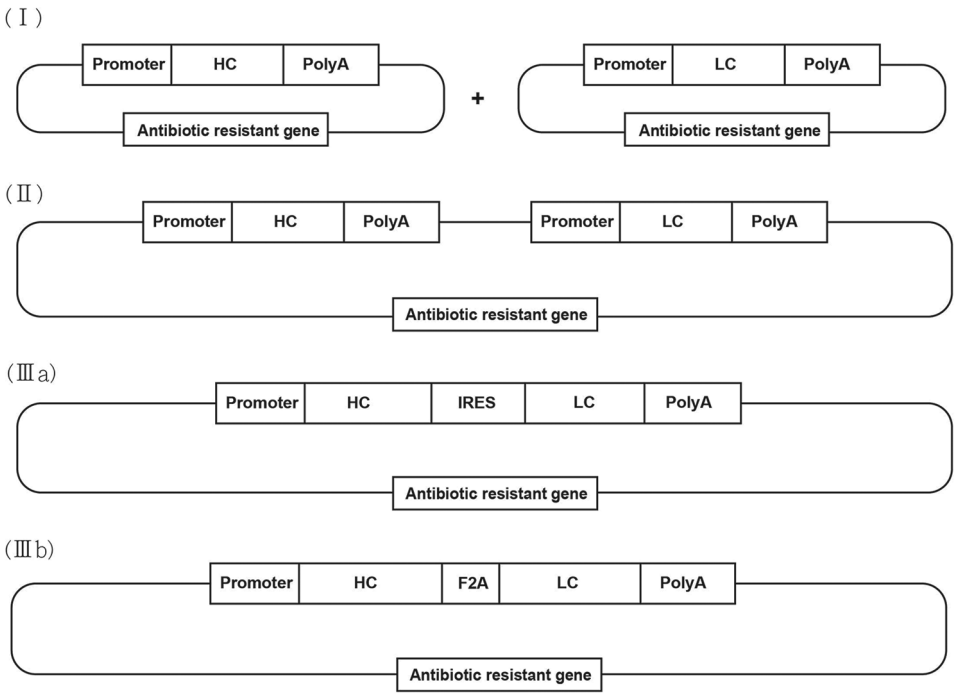

Figure 2: Schematic representation of expression vectors for recombinant monoclonal antibodies. (I) Monocistronic vectors, (II) Dual-promoter expression vector, (III) Bicistronic vectors: (a) IRES-mediated vector, (b) Furin-2A-mediated vector. HC: Heavy chain, LC: Light chain, PolyA: PolyA polyadenylation signal, IRES: Internal ribosomal entry, F2A: Furin-2A. Promoters commonly used are derived from the simian vacuolating virus 40, human cytomegalovirus immediate early promoter, and elongation factor-1α. The puromycin and blasticidin are widely used as selection antibiotics to establish the stable cell clones

IRES-mediated vector significantly, whether under transient or stable transfection conditions has been demonstrated by Ho *et al*. [36].

Transient expression

Achieving efficient transient expression of foreign genes in mammalian cells hinges on the successful delivery, cytoplasmic movement, and nuclear translocation of the introduced vector. However, several significant physicochemical obstacles hinder the delivery process both on the surface and inside the intended target cell. For instance, histone-modifying enzymes have been pinpointed as modulators of transgene expression from plasmid DNA. The application of inhibitors targeting these histone-modifying enzymes has been observed to amplify transgene expression levels [37].

Transient gene expression systems in mammalian cells are gaining popularity due to their ability to rapidly and efficiently produce substantial amounts of recombinant proteins. These systems offer scalability without the time-intensive processes required for establishing stable cell lines [38].

When producing rMAbs in CHO cells, the expression levels achieved with monocistronic vectors were found to be lower compared to dual-promoter vectors and bicistronic vectors [17]. The success of antibody yields for transient expression of monocistronic vectors is closely linked to the expression level of the LC chain, as an excess of LC facilitates more efficient folding and assembly of the rMAbs. Indeed, higher LC expression was demonstrated to correlate with higher cell viability, higher MAb productivity, and lower aggregation [39]. However, both the lengths of mRNA and protein of HC are twice of those of LC, when monocistronic vectors were used to express rMAb, the ratio of HC: LC of 3:2 would produce the optimal Mab titer [40].

Typically, 48 h after transfection, CHO cells were detached using trypsin, suspended in complete medium containing 10% fetal bovine serum (FBS), and then centrifuged at 1000 rpm for 5 min. The resulting pellet was washed thrice with phosphate-buffered saline. The cells were subsequently seeded in 6-well plates using CHO Pro Exp® serum-free medium with an equivalent number of cells for suspension-based expression. After 7 days, samples were collected and centrifuged at 13,000 rpm for 15 min at 4°C, and the supernatant was harvested for antibody expression analysis [9].

Conventionally, the production of rMAbs for preclinical evaluation through transient expression in CHO cells has been hindered by low titers. Enhancements in antibody production have been observed through the use of low temperatures and the addition of feed [33]. Curiously, suboptimal temperatures have also been shown to boost the transient expression of foreign genes in *E. coli* [41]. Moreover, advancements in cell engineering, elaborated on in the subsequent section, have the potential to further amplify antibody production.

Furthermore, the concurrent expression of nucleoside diphosphate kinase-A during transient expression has been shown to enhance transfection efficiency in CHO cells. This enhancement is likely attributed to the improved conveyance of plasmid DNA into the nucleus through the nuclear pore complex [42]. In addition, the integration of Epstein–Barr Nuclear Antigen-1 – a protein with the capacity to induce extrachromosomal maintenance – along with complementary oriP elements on a transient plasmid has been employed to mitigate the impact of plasmid dilution [43].

Establishment and selection of stable cell clones

Efficiently producing recombinant proteins, including antibodies, often requires screening an extensive array of clones to ultimately identify a stable and highly productive cell line [44]. Various selection methods have been developed to establish stable cells, with antibiotic-based selection systems such as puromycin (PURO) and blasticidin (BSD) being widely used.

For the expression of rMAbs, CHO cells were transfected using dual-promoter vectors, bicistronic vectors, or cotransfected with monocistronic vectors expressing HC and LC. Cells were treated with medium containing 8 μg/mL Puro and/or 15 μg/mL BSD 48 h after plasmid transfection. After 5–7 days, nontransfected cells were eliminated, and the selective medium was renewed every 2–3 days for 3 weeks to obtain stable cell pools. These cell pools were then expanded in selective medium containing 6 μg/mL Puro or 10 μg/mL BSD. Subsequently, distinct cell pools were seeded with an equal number of cells in 6-well plates for suspension expression. Supernatants were collected after 7 days for rMAbs expression analysis. Cell clones were derived using the limiting dilution method, diluting cells to 10 cells/mL in culture medium with 10% FBS and no screening pressure. The diluted solution was evenly distributed in a 96-well plate, with single clones identified 3–5 days later. On reaching 60% cell density, clones could be transferred to a 24-well plate for amplification culture.

In pursuit of more efficient stable cell establishment in CHO cells, alternative methods have been employed. The piggyBac transposon system, derived from the cabbage looper moth Trichoplusia, demonstrates superior transposition efficiency [45]. This system was adopted for expressing multiple genes in CHO cells [46]. In addition, the artificial chromosome expression (ACE) system leverages pre-engineered artificial chromosomes with recombination acceptor sites, facilitating targeted transfection of single or multiple genes without random integration into native host chromosomes [47]. ACE technology enabled the creation of candidate cell lines within 6 months, exhibiting expression levels surpassing 1 g/L and specific productivities up to 45 pg/cell/day under nonfed, nonoptimized shake flask conditions [48].

Notably, considerable expression variation among rMAbs produced by stably transfected CHO cells is well documented. Even within a single clone, expression heterogeneity can be remarkably high (standard deviation 50%–70% of the mean) [49]. Manipulating the ratio of HC/LC polypeptides to 1:2 or even 1:6 resulted in minimal high molecular weight aggregates and HC dimers. Consequently, optimizing

antibody engineering through pairing alternate LCs with HCs can enhance antibody expression and product quality when preserving or enhancing affinity [39,40].

Alongside antibiotic selection systems such as PURO, another approach involves the creation of dihydrofolate reductase-deficient CHO cells that can be cultivated and chosen using methotrexate-containing media. This technique allows for the generation of stable cell lines over a limited duration [50].

Moreover, the incorporation of the ubiquitous chromatin opening element (UCOES) sequence has demonstrated the ability to enhance antibody production in CHO cells [20]. UCOEs are characterized by their capacity to reliably provide stable transgene expression that is independent of the integration site and directly proportional to the copy number. Structurally, UCOEs comprised methylation-free CpG islands that enclose single or dual divergently transcribed promoters of housekeeping genes [51].

Various difficulties encountered

Numerous challenges have arisen during the expression of rMAbs in CHO cells. Certain obstacles might impact the effectiveness of these rMAbs and need to be addressed.

Aggregation

Given the potential to elicit immunogenic responses in both animals and humans, the control of antibody aggregates within therapeutic products is crucial [52]. When producing rMAbs using CHO cells, the resulting antibody aggregates can exhibit variation due to factors such as amino acid sequence, cell line characteristics, clonal diversity, and cell culture conditions [53]. The nature of antibody aggregates can differ based on solubility, covalent or noncovalent interactions, reversibility, and monomer denaturation. Notably, a lower culture temperature can amplify antibody aggregate formation in CHO cells with stably expressed rMAbs due to heightened transcription of LC and HC, coupled with constrained ER machinery [54]. Detection of these aggregates can be accomplished using size exclusion chromatography.

Numerous strategies have been devised to mitigate aggregation tendencies. For instance, incorporating trehalose, a disaccharide comprising two alpha-glucose units, into the medium has shown promise in avoiding aggregation [55]. Moreover, coexpressing chaperones, for example, disulfide isomerase protein disulfide isomerase (PDIa4), alongside antibodies can effectively curtail aggregate formation by averting misfolding of antibodies [56].

Posttranslational modifications

Glycosylation

The most common posttranslational modification of rMAbs involves glycosylation of the conserved asparagine residue in the CH2 domain (constant region of HC). Process changes during rMAb production often lead to variations in the oligosaccharide profile [57]. Extensively studied, the oligosaccharides commonly identified in rMAbs have been previously reviewed, detailing their effects on structure, stability, and biological functions [58,59]. Notably, particular glycoforms on rMAbs can wield substantial influence over their bioactivities and therapeutic characteristics [60]. For instance, high-mannose glycans can expedite antibody clearance rates, while afucosylation, bisecting GlcNAc residues, and high-mannose glycans can bolster antibody-dependent cell-mediated cytotoxicity [61,62]. Conversely, de-glycosylated IgG antibodies lack the capability to incite an *in vivo* inflammatory response [63]. As a result, the production of de-glycosylated rMAbs within CHO cells is preferable for therapeutic purposes [64,65].

Hydroxylation

Hydroxylation of lysine residues in rMAbs was observed in a consensus sequence (XKG), which is recognized as a modification site in other proteins like collagen. This hydroxylation was conjectured to arise from the activity of the CHO cell equivalent of the lysyl hydroxylase complex. This analogy with collagen and the presence of the consensus sequence led to the presumption that CHO cells employ similar enzymatic processes for lysine hydroxylation in rMAbs [66].

Genetic instability

A major challenge encountered in the industrial bioprocessing of rMAbs through engineered mammalian cells is the occurrence of cell line instability. This phenomenon involves a decline in the specific productivity of a production cell line. The instability of production can be attributed to two key mechanisms [67]: (1) epigenetic instability, characterized by methylation-induced transcriptional silencing of the promoter (such as CMV IE) responsible for driving the transcription of the rMAb gene and (2) genetic instability, manifested as a gradual reduction in the number of rMAb gene copies within a proliferating population of CHO cells.

Fragmentation

Fragmentation of rMAbs is a prevalent challenge impacting protein stability, necessitating vigilant oversight for essential quality control during the development of the production process. The presence of residual host cell proteases stands out as an important factor contributing to the fragmentation of rMAbs [68].

Disulfide reduction

A significant reduction in the inter-chain disulfide bonds of rMAbs produced within CHO cell culture can potentially occur during the harvesting procedures and/or the protein A chromatography stage [69,70]. This phenomenon may lead to the detection of antibody fragments encompassing the LC, HC, and diverse combinations of both chains.

Methionine oxidation

Oxidation of methionine (Met) residues stands as one of the prevalent pathways of protein degradation. In the context of rMAbs, oxidation of specific Met256 and Met432 residues has been identified to bring about changes in its interaction with protein A and protein G. This alteration results in a reduction in binding affinity between the rMAb and these proteins [71].

Frameshift

Frameshift mutations can lead to a complete change of the intended amino acid sequences, potentially impacting the biological functions of protein therapeutics and introducing

the risk of immunogenicity. A noteworthy instance involves the discovery and analysis of a novel-1 frameshift variant in a therapeutic IgG1 rMAb that was manufactured using CHO cells during the course of cell line selection investigations [72].

Culture conditions (medium; incubation temperature)

Over the past few decades, cell culture technology has experienced significant advancement and has transformed into a relatively dependable and resilient approach. A pivotal achievement in this progression was the creation and commercial availability of enhanced chemically defined media. Another notable breakthrough in media development was the transition toward serum-free formulations [73].

The optimization of medium composition has led to the suggestion of incorporating various chemicals to enhance cell culture conditions [74]. Among these, specific metabolites such as threonine and arachidonate have been proposed as potential additions to cell culture media [75]. Notably, sodium butyrate (NaBu) has gained attention for its ability to elevate the specific productivity of rMAbs in CHO cells [76]. This effect is attributed to NaBu's impact on the galactosylation of rMAbs, resulting in an improvement in the proper assembly of these antibodies [77].

Monoclonal antibodies are intricate heterogeneous glycoproteins undergoing multiple posttranslational modifications, leading to various variants, such as in charges, for a specific mAb. Charge variants are detectable through cation exchange chromatography, where the acidic mAb peak precedes the major peak, and the basic mAb peak follows. Conventionally, ion exclusion chromatography (IEC) has been utilized in mAb purification. A previous study investigated the impact of cell culture process control and ion supplementation on charge variants of mAbs produced by CHO cells. The study's findings revealed that under optimal conditions in 3 L bioreactors, significant enhancements were observed, particularly with Zn^{2+} and temperature shifts, which further improved antibody quality. The main peak saw a 12% increase, while the acidic peak decreased by 16%, with no notable loss in titer. These results provide valuable insights into methods for enhancing charge variants encountered during mAb production [16].

Furthermore, continuous perfusion has demonstrated the ability to maintain higher levels of cell density, product titer, and quality compared to intermittent perfusion culture. By combining high perfusion rates with a delayed reduction of culture temperature on day 6, the product titer reached a peak level of 16.19 g/L with a relative abundance of monomers at 97.6%. To validate the effectiveness of the optimized perfusion culture on a larger scale, a 200-L bioreactor was utilized, resulting in the highest product titer reaching 16.79 g/L by day 16. This study offers valuable insights for achieving highly efficient production of mAbs by CHO cells through an optimized perfusion culture strategy [78].

Host cell engineering

Host cell engineering has emerged as a strategy to enhance rMAbs production by manipulating specific gene expression in CHO cells [79]. The focus of many studies is apoptosis, a significant form of cell death, which has prompted the development of apoptosis-resistant cell lines through various tactics. These include approaches such as introducing anti-apoptotic Bcl-2-like proteins, suppressing pro-apoptotic factors, or inhibiting the p53 tumor suppressor protein [80]. Similarly, the overexpression of human telomerase reverse transcriptase in recombinant CHO cells has been pursued to foster proliferation and bolster resistance to apoptosis [81]. In addition, the overexpression of chaperones has been employed to facilitate the efficient maturation of recombinant and often intricate proteins or to mitigate the unfolded protein response in order to counteract apoptosis stemming from cellular stress [82].

Elevated expression of cell division cycle 25 homolog A within CHO cells has the potential to enhance traditional gene amplification systems through cell cycle manipulation during the initial phases of cell line development. Consequently, this manipulation leads to a swift augmentation in transgene copy count and a notable amplification of transgene expression [83].

Another challenge in heterologous protein expression revolves around the secretion pathway, beginning with the translocation of nascent polypeptide chains into the ER. Incorrect signal peptide cleavage can lead to aggregation of the LC. To mitigate this issue, the overexpression of SRP14, a component of the signal recognition particle, has been carried out, resulting in a substantial increase in the specific productivity of challenging-to-produce antibodies [84].

Furthermore, transient overexpression of PDI has been demonstrated to elevate the rate of rMAb secretion [85]. In addition, the positive influence of two miRNAs (miR-557 and miR-1287) on viable cell density and specific productivity in rMAb-expressing CHO cells has been established [86].

For the purpose of enhancing the affinity of rMAbs through increased mutation, several strategies have been explored. Activation-induced cytidine deaminase (AID) initiates somatic hypermutation by converting deoxycytidines to deoxyuracils, thereby inducing additional mutations. AID plays a pivotal role in diversifying the antibody repertoire in B cells. The introduction of a plasmid expressing AID into CHO cells has been employed to heighten affinity, specificity, and stability of the produced antibodies [87,88].

Factors affecting recombinant monoclonal antibody yield

In addition to CHO cells, a variety of systems have been employed for generating recombinant proteins, including both transient and stable expression methods. It might be valuable to explore the possibility of adapting conditions utilized in other cell types for application in CHO cells.

Effect of 2-aminopurine on recombinant monoclonal antibody production in Chinese hamster ovary cells

The specific effect of 2-aminopurine (2-AP) on rMAb production in CHO cells is not known. However, in human cell lines, 2-AP has been shown to enhance the transient expression of exogenous genes [89]. It is possible that similar

effects could be observed in CHO cells, potentially leading to increased transient expression of rMAbs. Further research would be needed to determine the exact impact of 2-AP on rMAb production in CHO cells.

Effect of protein kinase R and ribonuclease L on transient expression of recombinant monoclonal antibody production in Chinese hamster ovary cells

Protein kinase R (PKR) and 2'-5'oligoadenylate-dependent ribonuclease L (RNase L) are intracellular enzymes known to restrict viral replication in interferon-treated cells and can suppress transient expression of heterologous genes [90]. The effect of PKR and RNase L on transient expression of rMAb production in CHO cells would likely involve similar suppression of transient gene expression. Their presence could potentially reduce the efficiency of rMAb production during transient expression. Then, the transient expression of rMAbs will probably be enhanced in PKR knockout and/or RNase L knockout CHO cells.

Effect of adenosine deaminase acting on RNA 1 on transient expression of recombinant monoclonal antibody production in Chinese hamster ovary cells

The information suggests that coexpression of adenosine deaminase acting on RNA 1 (ADAR1) can enhance plasmid-based gene expression in various cell lines [91]. This enhancement seems to be independent of promoter type, protein reporter, and cell line. Therefore, it is plausible that coexpression of ADAR1 in CHO cells could similarly enhance the transient expression of rMAbs by increasing gene expression efficiency. However, as with any experimental manipulation, actual effects would need to be validated in the specific context of rMAb production in CHO cells.

Effect of Bcl-xL expression on transient and stable expression of recombinant monoclonal antibodies

The information suggests that transient expression of the Bcl-xL anti-apoptotic protein can extend cell survival and protect cells from apoptosis [80]. While this effect could potentially enhance transient expression of rMAbs by increasing cell viability, it might not necessarily directly facilitate stable expression. Stable expression involves different mechanisms, including integration of the transgene into the host cell genome, which may not be directly influenced by anti-apoptotic factors like Bcl-xL. Further investigation would be needed to assess the impact of Bcl-xL expression on both transient and stable expression of rMAbs.

Effect of protein disulfide isomerase expression on transient and stable expression of recombinant monoclonal antibodies

The information suggests that transient overexpression of PDI can increase the secretion rate of rMAbs [85]. Similar to Bcl-xL, the effect of PDI on stable expression might differ due to the distinct mechanisms involved. While PDI could potentially enhance the secretion efficiency of rMAbs during transient expression, stable expression relies on various factors, including chromosomal integration and long-term maintenance of transgene expression. Additional investigations would be needed to determine the impact of PDI expression on both transient and stable expression of rMAbs in CHO cells.

Effect of temperature on recombinant monoclonal antibody production in Chinese hamster ovary cells

Improvements in the transient production of rMAbs have been noted by implementing lower temperatures in CHO cells [33,92]. Conversely, a reduced culture temperature has the potential to increase the formation of antibody aggregates in CHO cells with stably expressed rMAbs [54]. Investigating the molecular mechanisms underlying the distinctions between transient and stable expression promises to be a captivating endeavor.

Effect of synthetic 5'-untranslated region on recombinant monoclonal antibody production in Chinese hamster ovary cells

Three synthetic 5'-UTRs that enhance protein expression from the CMV promoter have been discovered using a high-throughput strategy involving the design, screening, and optimization of 5' UTRs [93]. The impact of these synthetic 5'-UTRs on rMAb production in CHO cells warrants further investigation.

Effect of 3'-untranslated region on recombinant monoclonal antibody production in Chinese hamster ovary cells

Beyond the 5' cap, 5'-UTRs, coding region, and polyadenylation tail, the 3'-UTR plays a crucial role in mitigating the immunogenicity of mRNA vaccines, ensuring consistent improvement in intracellular stability and translational efficiency [29]. Both the 5'-UTR and the downstream 3'-UTR contribute significantly to the stability of mRNA vaccines [30]. Therefore, exploring the influence of 3'-UTRs on rMAb production in CHO cells is a subject that merits further investigation.

Conclusion

Indeed, CHO cells have been the predominant choice for scientific research and industrial production of rMAbs since their inception, with approximately 75% of related scientific publications consistently employing these cells. This steady percentage over the past decade suggests that CHO cells are likely to continue playing a pivotal role in protein expression within mammalian cell culture for the foreseeable future. Despite their long-standing history, ongoing efforts are directed toward refining the production of rMAbs in CHO cells [11].

Advancements in multiple areas are contributing to the continuous improvement of rMAb production in CHO cells. These include the refinement of medium formulations to optimize culture conditions, the development of more efficient expressing vectors to enhance gene expression, and the exploration of diverse cell engineering technologies aimed at augmenting rMAb expression levels in CHO cells [11,18]. As the demand for rMAbs grows and their applications diversify, research and development efforts are poised to further advance the capabilities and potential of CHO cell-based protein expression platforms.

Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflicts of interest

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