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From omics to Cellular mechanisms in mammalian cell factory development

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Abstract

Mammalian cells have been used widely as biopharmaceutical cell factories due to their ability to make complex biotherapeutic proteins with human-compatible modifications. However, their application for some products has been hampered by low protein yields. Numerous studies have aimed to characterize cellular bottlenecks in the hope of boosting protein productivity, but the complexity of the underlying pathways and the diversity of the modifications have complicated cell engineering when relying solely on traditional methodologies. Incorporating omics-based and systems approaches into cell engineering can provide valuable insights into desirable phenotypes of cell factories. Here, we discuss cell engineering strategies for enhancing protein productivity in mammalian cell factories, particularly CHO and HEK293, and the opportunities and limitations of the genome-wide screening and multi-omics approaches for guiding cell engineering. Systems biology strategies will also be discussed to show how they refine our understanding of the cellular mechanisms which will aid in effective engineering strategies.

Keywords

Cell engineering; Omics technologies; Systems biology; Protein productivity

Introduction

Many diseases result from dysfunction of specific proteins; thus, therapeutic proteins provide breakthrough therapies for diverse diseases. For example, monoclonal antibodies with cancer-targeting properties have revolutionized immunotherapy. However, the rising demand for high-quality recombinant therapeutics requires efficient cell–based

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manufacturing. Thus, there has been a need to develop new host cells by engineering cellular pathways involved in cell viability, high cell density, and specific productivity. In particular, the host cell secretory pathway is a common bottleneck in the production of many difficult to express (DTE) recombinant proteins (rProtein). The overexpression of a heterologous protein can overwhelm the secretory pathway, leading to cell stress, inefficient secretion, and protein aggregation, ultimately triggering apoptosis. Thus, engineering the secretory pathway has been of interest in many cell engineering strategies to alleviate bottlenecks. Recent advances in high throughput omics technologies, genome wide screening, and systems biology now provide invaluable tools for investigating host cell traits linked to protein production and elucidate novel engineering targets to boost cell productivity. Here, we first discuss the two most common engineering strategies for enhancing protein yield: improving the cell viability and improving specific productivity. We then highlight advances in genome-wide screening (CRISPR and RNAi screening) and omics-based technologies (genomics, epigenomics, transcriptomics, proteomics, and interactomics) used to enhance protein productivity. Finally, we discuss how advances in systems biology strategies can elucidate cellular mechanisms involved in protein production and introduce effective engineering strategies.

Improving host cells by enhancing culture longevity and cell viability

In bioreactors, apoptosis can be triggered by different types of stress stemming from hypoxia, shear, nutrient depletion, temperature, protein misfolding, osmolality, and pH. Apoptosis engineering targets genes such as the BCL2 family, caspases, heat shock proteins, and cell cycle components, resulting in apoptosis-resistant cell lines with extended lifespan and higher viable cell densities [1,2]. For example, multiplexed CRISPR-Cas9 was used to remove pro-apoptotic Bak, Bax, and Fut8, leading to knockout cells with improved resistance to apoptosis [3]. Similarly, overexpressing HSP27, an anti-apoptosis heat shock protein, in monoclonal antibody (mAb)-expressing CHO cells boosted viable cell density, delayed loss of culture viability, and increased mAb titer without affecting N-glycosylation by slowing down caspase activities [4]. As another example, mTOR, a kinase regulating cell cycle and proliferation, was overexpressed, extending culture duration by up to 4 days, leading to 2–3 fold increases in IgG titer [5]. In HEK293 cells, four pro-apoptotic genes were knocked out (Caspase-3, Caspase-6, Caspase-7 and AIF1), which generated an apoptosis-resistant HEK293 cell line with increased expression of recombinant apoptosis-inducing proteins [6]. With the aim of engineering a high-producing host for efficient transient expression, Bax and Bak were knocked out in HEK293 cells. This double knock-out increased resistance to apoptosis and shear stress, compared to the parental cells [7]. Additionally, several nutrients are converted to growth inhibitory byproducts during cell culture, such as lactate and ammonia [8]. Various metabolic engineering approaches have reduced toxic byproduct buildup in bioreactors [9]. For example, Mulukutla et al. engineered phenylalanine-tyrosine (Phe-Tyr) and branched chain amino acid (BCAA) catabolic pathways of CHO cells and significantly improved cell culture process performance [10].

Improving host cells by boosting specific productivity

In addition to gains from enhanced cell viability, increased biotherapeutic yields can be achieved by increasing cell specific productivity. Towards this, engineering strategies have targeted different components and subpathways of the secretory pathway, e.g., chaperones, protein disulfide isomerases (PDIs), protein trafficking, and the UPR. For example, intracellular chaperone retention rates in the ER were improved, boosting productivity. Specifically, overexpression of the KDEL receptor (KDELR), which mediates localization of soluble ER chaperones, enhanced specific productivity of IgG by 13.2% during exponential phase and 23.8% in deceleration phase in CHO batch culture experiments. [11]. In another study, stable downregulation of two secretory pathway genes, Tbc1D20 and CerS2, in CHO-IgG-producing cells increased specific productivity by 50–66%. [12]. Furthermore, the benefits were neither cell line nor productspecific, as the same combined knockdown in CHO-DG44 cells stably expressing a distinct IgG antibody and human serum albumin showed significant improvements in specific productivity. Another group developed a high-throughput microscale transfection platform to simultaneously target and test multiple genes encoding discrete ER or secretory pathway components [13]. Improvements in cell growth were seen with an even greater impact on specific productivity. This benefit may stem from improved vesicular trafficking at the ER/Golgi complex interface. Finally, specific productivity was also improved in HEK293 cells through folding and secretory pathway engineering. For example, overexpression of SNAP-23 (synaptosome-associated protein of 23 kDa) and VAMP8 (vesicleassociated membrane protein 8), involving in the fusion of secretory vesicles to the plasma membrane, increased the production of recombinant proteins in HEK293 cell [14].

Genome-wide genetic screens provide actionable engineering targets

Genome-wide screens using RNAi or CRISPR-Cas9 are popular, high-throughput techniques that have identified novel cell engineering targets (Figure 1) [15]. For instance, a cross-species screen in CHO cells using a mouse siRNA library identified targets for enhancing rProtein titer, including knockdown of Rad21 (from the cohesin complex) and Chd4 (from the histone deacetylase NuRD complex) [16]. Another CHO- specific RNAi screen identified genes that consistently enhance antibody production, including Cyp1a2, Atp5s, and Dgki genes (involved in distinct pathways and localized in different organelles). Targeting these genes resulted in >90% increase in specific antibody productivity [17]. Strategies for engineering HEK293 cells also were found using screens, such as an unbiased genome-wide siRNA screen developed by Inwood et al. [18]. As an example, Xiao et al. identified target genes associated with recombinant firefly luciferase expression in HEK293 and demonstrated they were in several pathways involved in spliceosome formation and mRNA processing, transcription, metabolic processes, transport, and protein folding. Among the confirmed genes, silencing OAZ1, the gene encoding the ornithine decarboxylase antizyme1, was shown to enhance luciferase expression without affecting cell viability [19].

Aside from protein titer, screens have found targets for other important traits. For example, the metabolite glutamine is usually over-fed to increase TCA cycle flux. However, ammonia may accumulate and inhibit growth, so a cell-line capable of growing in glutamine-free

media would be valuable. A CRISPR-Cas9 screen in CHO cells identified a network of glutamine-sensitive genes, which included Abhd11, a poorly characterized lipase. Its deletion substantially improved cell growth in glutamine-free medium [20]. Another study exploited genome-wide CRISPR screens to identify innate immune and metabolic restriction factors that limit cell-derived influenza vaccine production. This included 64 putative influenza restriction factors wherein GO enrichment showed highly significant enrichment of terms related to influenza, but the majority of the putative restriction factors identified were not associated with these GO terms [21]. Thus, genome-wide screens can provide novel engineering targets (Figure 1); however, further knowledge is needed to fully describe the molecular mechanisms through which these targets contribute to desired phenotypes. The generation of additional omics data combined with systems modeling efforts can provide the needed insight into these mechanisms to make target selection more robust.

Omics-based profiling of host cells

To further our understanding of the cellular biology of rProtein production, many studies have leveraged omics data at the genome, transcriptome, proteome, and metabolome levels (Figure 2). The publication of the CHO-K1 genome [22] laid the foundation for CHO systems biology research. Analysis of additional CHO cell line genomes have further facilitated the targeted engineering for biopharmaceutical production and application of other omics technologies [23,24]. For example, an analysis of the CHO-K1 genome found homologs to 99% of human glycosyltransferases; however only 53% of these genes are expressed in CHO [22]. Such genomic studies can guide the targeted engineering of glycosylation genes to improve biopharmaceutical production in CHO cell lines [25]. Additionally, research in CHO genome stability has been facilitated by the ongoing efforts to sequence commonly used CHO cell lines. Spahn and Zhang et al. [26] leveraged whole-genome sequencing data from 11 CHO cell lines and identified mutations in key DNA repair genes in CHO. The authors subsequently reverted these mutations through genome editing and demonstrated improved DNA repair efficiency and reduced chromosome instability, resulting in significantly increased stability of product titer in long-term cultures. Importantly, an accurate and complete reference genome sequence enables cell engineering efforts, which is being achieved by efforts to completely resequence *C. griseus* using the hybrid assembly with single molecule real time and short read Illumina- based sequencing [27], along with high- throughput chromosome conformation capture methods [28].

Transcriptomics studies have also helped determine gene targets for cell line engineering by leveraging analysis techniques such as differential expression analysis, gene ontology, and pathway enrichment. For example, transcriptomics helped identify 32 genes that are consistently upregulated in high producing CHO cell clones [29], suggesting these genes may play a role in mediating high productivity. An ontology analysis revealed that these candidate genes were predominantly involved in signaling, protein folding, cytoskeleton organization, and cell survival functions. They found several targets that showed beneficial effects, most notably overexpression of Foxa1 resulted in increased cell density, viability, and yield of difficult-to express proteins [29]. Another group conducted a meta-analysis of CHO transcriptomic datasets to identify engineering targets to improve rProtein production [30].

Integrating additional omics can shed further light (Figure 2). For example, integrating transcriptomics, metabolomics and fluxomics showed that a broad adaptation of the cellular network compensated resources for recombinant protein production while maintaining the same growth rate in HEK293 cells. [31]. This study also showed upregulation of genes associated with endoplasmic reticulum stress indicates a possible bottleneck at the point of protein folding and assembly. In another study, the authors analyzed genomic and transcriptomic data, along with metabolic pathway analysis, from industrial HEK293 variants and parental cells. Changes were found in the expression of cellular component organization, cell motility and cell adhesion. Specifically, alterations in gene expression between adherent and suspension included changes in cholesterol biosynthesis and other processes during the transition from adherent to suspension growth [32].

Since the correlation between mRNA and protein abundance is not always linear, many genomic and transcriptomic studies have been supported with complementary proteomic analyses. Proteomic technologies have quantified different proteins in high- and low-producing CHO cell lines, enabling identification of protein factors potentially significant for protein productivity. For example, comparative proteomics of three CHO sublines identified regulators of cell growth and protein expression [33], ultimately proposing novel engineering strategies to optimize CHO host cells.

In addition to the more common forms of omics technologies, researchers have also applied metabolomics [34], lipidomics [35–37], epigenomics [23], and glycomics [38] to investigate mammalian host cell biology and reveal novel engineering targets. However, while the diverse omics technologies have been invaluable for profiling the cells and providing novel biological insights, the molecular mechanisms driving enhanced productivity still remain unclear. Interactomics could potentially elucidate mechanisms by identifying interactions between molecules, allowing us to directly capture the molecular pathways underlying different cellular phenomena, including protein secretion [39]. While high-throughput efforts have charted a significant portion of the human protein interactome [40], weaker and more transient interactions can often escape detection using standard methods such as co-immunoprecipitation. Since many transient interactions support protein secretion [41–45], novel proximity-based labeling techniques [46] have been applied to profile the dynamic interactions between recombinant secreted protein and the secretory pathway. For example, BioID, a method wherein a BirA domain is fused to the protein of interest, can biotinylate all interacting proteins. Using BioID, protein interaction networks were found that support the synthesis and secretion of SERPIN-family proteins, each bearing different post-translational modifications. The effort led to identification of oxidative folding as a cellular bottleneck and a specific disulfide isomerase (PDIA4) as a crucial enzyme for expression of disulfide bond-rich rProteins [47]. PDIA4 was also found to support monoclonal antibodies production in CHO cells [48], supporting its crucial role in secretion of molecules with multiple disulfide bonds. While these methods help generate the omics data of the future, without cutting edge modeling to understand the underlying connections we cannot create a complete picture of the interactome (Figure 2).

Systems approaches supplement cell engineering by elucidating molecular mechanisms.

While omics data are shedding light on rProtein production in mammalian cells, many omics findings only provide a snapshot of the cellular mechanisms. More recently, efforts to integrate insights from omics studies with mechanistic knowledge of the mammalian host can provide more detailed insights into mechanisms underlying the phenotypes being studied (Figure 2). A recent study identified and validated key genes underlying high-density suspension HEK293 cell lines by taking a network approach to multi-omics data analysis [32]. For CHO cells, genome-scale metabolic models (GeM) [49–52] have accelerated the integrative analysis and interpretation of omics data by accounting for cellular context and biochemical constraints (e.g., metabolic flux balancing and mass conservation), and is enabling more targeted cell line engineering by bridging genotype and phenotype [53]. For example, an integrative analysis coupling GeM with omics data [54] identified significantly altered pathways during antibody production, including energy metabolism, extracellular transport, and amino acid metabolism. To identify genes characteristic of high mAb production, studies have paired GeMs with transcriptomic data [55] and enzyme assays [56]. GeMs have also been extended to the CHO secretory pathway [57,58], enabling the prediction of metabolic costs associated with protein secretion. Furthermore, the model also allows the simultaneous quantification of other secreted host cell proteins. By knocking out several host cell proteins that impose heavy secretory energetic burden, improved growth characteristics were obtained in the engineered cells [59].

GeMs have also helped identify targets to engineer in microbial species by simulating the effects of gene deletion, addition, and modulation of expression levels [60–62]. While, the complexity of mammalian cells has limited similar applications in CHO, hybrid models, combining data-driven approaches with mechanistic insights, provide a middle ground between the two types of models, allowing for more realistic parameterization of the otherwise complex, underdetermined GeMs [63,64]. For example, metabolite analysis was coupled with metabolic models to optimize fed-batch cultivations of CHO cells [65]. When augmented by machine learning, CHO metabolic models were shown to accurately predict time course dependent prediction of individual amino acid concentration in culture medium throughout the production process [66]. These approaches integrating mechanistic models with machine learning will be invaluable for increasing the predictive nature of GeMs by addressing uncertainty among some pathways.

Conclusion:

Advances in omics and genome editing tools have helped in the study and development of cell lines for rProtein production. However, the impact of omics technologies can be greatly increased when accompanied by systems approaches to elucidate the molecular mechanisms underlying desired phenotypes in biotherapeutic production. While systems approaches often shed light on engineering targets within the cell, the resulting titer improvements reported by the studies surveyed in this review [59,62] remained moderate in contrast with traditional approaches such as media and bioprocess optimization, clonal

selection, and bioreactor designs [67]. We argue that advances in omics data generation and sharing will help augment current modeling techniques, allowing for more context-specific predictions and engineering target discovery. As omics data are integrated with genome-wide screens, phenotypic data (e.g., specific productivity and/or cell culture longevity) and systems approaches, a clearer image of the protein secretion and associated pathways will be achieved, thus allowing the generation of custom producers and an expanded list of engineering targets. Additionally, identification of the protein interaction networks supporting biosynthesis of rProteins helps define the product- specific secretion machinery, opening avenues for mammalian cell engineering efforts, wherein biotherapeutic production hosts can be rationally engineered to improve the titer of diverse proteins in a client- specific manner.

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Reference:

1. Grilo AL, Mantalaris A. Apoptosis: A mammalian cell bioprocessing perspective. Biotechnol Adv. 2019;37: 459–475. doi:10.1016/j.biotechadv.2019.02.012 [PubMed: 30797096]
2. Henry MN, MacDonald MA, Orellana CA, Gray PP, Gillard M, Baker K, et al. Attenuating apoptosis in Chinese hamster ovary cells for improved biopharmaceutical production. Biotechnol Bioeng. 2020;117: 1187–1203. doi:10.1002/bit.27269 [PubMed: 31930480]
3. Grav LM, Lee JS, Gerling S, Kallehauge TB, Hansen AH, Kol S, et al. One-step generation of triple knockout CHO cell lines using CRISPR/Cas9 and fluorescent enrichment. Biotechnol J. 2015;10: 1446–1456. doi:10.1002/biot.201500027 [PubMed: 25864574]
4. Tan JGL, Lee YY, Wang T, Yap MGS, Tan TW, Ng SK. Heat shock protein 27 overexpression in CHO cells modulates apoptosis pathways and delays activation of caspases to improve recombinant monoclonal antibody titre in fed-batch bioreactors. Biotechnol J. 2015;10: 790–800. doi:10.1002/biot.201400764 [PubMed: 25740626]
5. Dreesen IAJ, Fussenegger M. Ectopic expression of human mTOR increases viability, robustness, cell size, proliferation, and antibody production of chinese hamster ovary cells. Biotechnol Bioeng. 2011;108: 853–866. doi:10.1002/bit.22990 [PubMed: 21404259]
6. Zhang W, Xiao D, Shan L, Zhao J, Mao Q, Xia H. Generation of apoptosis-resistant HEK293 cells with CRISPR/Cas mediated quadruple gene knockout for improved protein and virus production. Biotechnol Bioeng. 2017;114: 2539–2549. doi:10.1002/bit.26382 [PubMed: 28710851]
7. Arena TA, Chou B, Harms PD, Wong AW. An anti-apoptotic HEK293 cell line provides a robust and high titer platform for transient protein expression in bioreactors. MAbs. 2019;11: 977–986. doi:10.1080/19420862.2019.1598230 [PubMed: 30907238]
8. Pereira S, Kildegaard HF, Andersen MR. Impact of CHO Metabolism on Cell Growth and Protein Production: An Overview of Toxic and Inhibiting Metabolites and Nutrients. Biotechnol J. 2018;13: e1700499. doi:10.1002/biot.201700499 [PubMed: 29393587]
9. Richelle A, Lewis NE. Improvements in protein production in mammalian cells from targeted metabolic engineering. Curr Opin Syst Biol. 2017;6: 1–6. doi:10.1016/j.coisb.2017.05.019 [PubMed: 29104947]
10. Mulukutla BC, Mitchell J, Geoffroy P, Harrington C, Krishnan M, Kalomeris T, et al. Metabolic engineering of Chinese hamster ovary cells towards reduced biosynthesis and accumulation of novel growth inhibitors in fed-batch cultures. Metab Eng. 2019;54: 54–68. doi:10.1016/j.ymben.2019.03.001 [PubMed: 30851381]

11. Samy A, Kaneyoshi K, Omasa T. Improvement of Intracellular Traffic System by Overexpression of KDEL Receptor 1 in Antibody-Producing CHO Cells. Biotechnol J. 2020;15: e1900352. doi:10.1002/biot.201900352 [PubMed: 32073237]

12. Pieper LA, Strotbek M, Wenger T, Gamer M, Olayioye MA, Hausser A. Secretory pathway optimization of CHO producer cells by coengineering of the mitosRNA-1978 target genes CerS2 and Tbc1D20. Metab Eng. 2017;40: 69–79. doi:10.1016/j.ymben.2017.01.003 [PubMed: 28088541]

13**. Cartwright JF, Arnall CL, Patel YD, Barber NOW, Lovelady CS, Rosignoli G, et al. A platform for context-specific genetic engineering of recombinant protein production by CHO cells. J Biotechnol. 2020;312: 11–22. doi:10.1016/j.jbiotec.2020.02.012 [PubMed: 32114154] This study describes the development of a high-throughput microscale platform that can perform multi-parallel testing of various genetic components. They demonstrate the utility of this platform by simultaneously evaluating the functional effect of 32 ER or secretory pathway genes in different combinations and varying levels of expression in CHO-K1 MAb-A producing cell lines. They were able to achieve improvements in both cell viability and specific productivity using this novel platform.

14. Peng R-W, Abellan E, Fussenegger M. Differential effect of exocytic SNAREs on the production of recombinant proteins in mammalian cells. Biotechnol Bioeng. 2011;108: 611–620. doi:10.1002/bit.22986 [PubMed: 21246508]

15. Lee JS, Grav LM, Lewis NE, Faustrup Kildegaard H. CRISPR/Cas9-mediated genome engineering of CHO cell factories: Application and perspectives. Biotechnol J. 2015;10: 979–994. doi:10.1002/biot.201500082 [PubMed: 26058577]

16. Klanert G, Fernandez DJ, Weinguny M, Eisenhut P, Bühler E, Melcher M, et al. A cross-species whole genome siRNA screen in suspensioncultured Chinese hamster ovary cells identifies novel engineering targets. Sci Rep. 2019;9: 8689. doi:10.1038/s41598-019-45159-2 [PubMed: 31213643]

17. Lin P, Liu R, Alvin K, Wahyu S, Murgolo N, Ye J, et al. Improving Antibody Production in Stably Transfected CHO Cells by CRISPR-Cas9 Mediated Inactivation of Genes Identified in a Large-Scale Screen with Chinese Hamster- Specific siRNAs. Biotechnology Journal. 2020. p. 2000267. doi:10.1002/biot.202000267

18. Inwood S, Betenbaugh MJ, Lal M, Shiloach J. Genome-Wide High-Throughput RNAi Screening for Identification of Genes Involved in Protein Production. Methods Mol Biol. 2018;1850: 209–219. doi:10.1007/978-1-4939-8730-6_14 [PubMed: 30242689]

19. Xiao S, Chen YC, Buehler E, Mandal S, Mandal A, Betenbaugh M, et al. Genome-scale RNA interference screen identifies antizyme 1 (OAZ1) as a target for improvement of recombinant protein production in mammalian cells. Biotechnol Bioeng. 2016;113: 2403–2415. doi:10.1002/bit.26017 [PubMed: 27215166]

20*. Karottki KJ la C, Hefzi H, Li S, Pedersen LE, Spahn PN, Joshi C, et al. A metabolic CRISPR-Cas9 screen in Chinese hamster ovary cells identifies glutamine-sensitive genes. Metab Eng. 2021;66: 114–122. doi:10.1016/j.ymben.2021.03.017 [PubMed: 33813034] This study found that knockout of a novel engineering target Abhd11 resulted in increased CHO-S cell growth in glutamine-free media presumably by impacting the TCA cycle. This target was identified using a pooled CRISPR/Cas9 library targeting genes involved in CHO-specific metabolism and subsequent CHO CRISPR knockout library cells screening for growth in media with and without glutamine.

21*. Sharon DM, Nesdoly S, Yang HJ, Gélinas J-F, Xia Y, Ansorge S, et al. A pooled genome-wide screening strategy to identify and rank influenza host restriction factors in cell-based vaccine production platforms. Sci Rep. 2020;10: 12166. doi:10.1038/s41598-020-68934-y [PubMed: 32699298] A genome-wide knockout pooled CRISPR/Cas9 library coupled to a FACS selection screening strategy were used to identify punitive restriction factors corresponding to viral titer of influenza in HEK-293SF cells. This strategy allowed for identification of potential engineering targets for the creation of high yield knockout cell lines for influenza vaccine production.

22. Xu X, Nagarajan H, Lewis NE, Pan S, Cai Z, Liu X, et al. The genomic sequence of the Chinese hamster ovary (CHO)-K1 cell line. Nat Biotechnol. 2011;29: 735–741. doi:10.1038/nbt.1932 [PubMed: 21804562]

23. Feichtinger J, Hernández I, Fischer C, Hanscho M, Auer N, Hackl M, et al. Comprehensive genome and epigenome characterization of CHO cells in response to evolutionary pressures and over time. Biotechnol Bioeng. 2016;113: 2241–2253. doi:10.1002/bit.25990 [PubMed: 27072894]

24. Brinkrolf K, Rupp O, Laux H, Kollin F, Ernst W, Linke B, et al. Chinese hamster genome sequenced from sorted chromosomes. Nat Biotechnol. 2013;31: 694–695. doi:10.1038/nbt.2645 [PubMed: 23929341]

25. Yang Z, Wang S, Halim A, Schulz MA, Frodin M, Rahman SH, et al. Engineered CHO cells for production of diverse, homogeneous glycoproteins. Nat Biotechnol. 2015;33: 842–844. doi:10.1038/nbt.3280 [PubMed: 26192319]

26. Spahn PN, Zhang X, Hu Q, Hamaker NK, Hefzi H, Li S, et al. Restoration of deficient DNA Repair Genes Mitigates Genome Instability and Increases Productivity of Chinese Hamster Ovary Cells. Cold Spring Harbor Laboratory. 2021. p. 2021.01.07.425558. doi:10.1101/2021.01.07.425558

27*. Rupp O, MacDonald ML, Li S, Dhiman H, Polson S, Griep S, et al. A reference genome of the Chinese hamster based on a hybrid assembly strategy. Biotechnol Bioeng. 2018;115: 2087–2100. doi:10.1002/bit.26722 [PubMed: 29704459] Using hybrid single molecule real time sequencing merged with Illumina-based assemblies authors generated a more contiguous and complete genome assembly than either technology alone, reducing the number of scaffolds by >28-fold, with 90% of the sequence in the 122 longest scaffolds and facilitate the genome engineering efforts.

28. Hilliard W, MacDonald ML, Lee KH. Chromosome- scale scaffolds for the Chinese hamster reference genome assembly to facilitate the study of the CHO epigenome. Biotechnology and Bioengineering. 2020. pp. 2331–2339. doi:10.1002/bit.27432 [PubMed: 32410221]

29. Berger A, Le Fourn V, Masternak J, Regamey A, Bodenmann I, Girod P-A, et al. Overexpression of transcription factor Foxa1 and target genes remediate therapeutic protein production bottlenecks in Chinese hamster ovary cells. Biotechnol Bioeng. 2020;117: 1101–1116. doi:10.1002/bit.27274 [PubMed: 31956982]

30*. Tamošaitis L, Smales CM. Meta-Analysis of Publicly Available Chinese Hamster Ovary (CHO) Cell Transcriptomic Datasets for Identifying Engineering Targets to Enhance Recombinant Protein Yields. Biotechnol J. 2018;13: e1800066. doi:10.1002/biot.201800066 [PubMed: 29917317] This study performs a Mata-Analysis of CHO transcriptomic data to identify common pathways and genes underlying cell growth and product yield. They find that only the cell cycle and lysosome pathway show good concordance; mapping out the contributing genes to ultimately construct a transcriptomic ‘fingerprint’ of a high-performing CHO cell line.

31. Dietmair S, Hodson MP, Quek L-E, Timmins NE, Gray P, Nielsen LK. A multi-omics analysis of recombinant protein production in Hek293 cells. PLoS One. 2012;7: e43394. doi:10.1371/journal.pone.0043394 [PubMed: 22937046]

32. Malm M, Saghaleyni R, Lundqvist M, Giudici M, Chotteau V, Field R, et al. Author Correction: Evolution from adherent to suspension: systems biology of HEK293 cell line development. Sci Rep. 2021;11: 5407. doi:10.1038/s41598-021-85105-9 [PubMed: 33654173]

33. Xu N, Ma C, Ou J, Sun WW, Zhou L, Hu H, et al. Comparative Proteomic Analysis of Three Chinese Hamster Ovary (CHO) Host Cells. Biochem Eng J. 2017;124: 122–129. doi:10.1016/j.bej.2017.05.007 [PubMed: 28736500]

34. Mulukutla BC, Kale J, Kalomeris T, Jacobs M, Hiller GW. Identification and control of novel growth inhibitors in fed-batch cultures of Chinese hamster ovary cells. Biotechnol Bioeng. 2017;114: 1779–1790. doi:10.1002/bit.26313 [PubMed: 28409820]

35. Budge JD, Knight TJ, Povey J, Roobol J, Brown IR, Singh G, et al. Engineering of Chinese hamster ovary cell lipid metabolism results in an expanded ER and enhanced recombinant biotherapeutic protein production. Metab Eng. 2020;57: 203–216. doi:10.1016/j.ymben.2019.11.007 [PubMed: 31805379]

36. Yeo HC, Chen S, Ho YS, Lee D-Y. An LC-MS-based lipidomics preprocessing framework underpins rapid hypothesis generation towards CHO systems biotechnology. Metabolomics. 2018;14: 98. doi:10.1007/s11306-018-1394-0 [PubMed: 30830409]

37. High-Throughput Lipidomic and Transcriptomic Analysis To Compare SP2/0, CHO, and HEK-293 Mammalian Cell Lines. Anal. Chem 2017; 89: 1477–1485. doi:10.1021/acs.analchem.6b02984.s001 [PubMed: 27991764]

38. Sumit M, Dolatshahi S, Chu A-HA, Cote K, Scarcelli JJ, Marshall JK, et al. Dissecting N-Glycosylation Dynamics in Chinese Hamster Ovary Cells Fed-batch Cultures using Time Course Omics Analyses. iScience. 2019;12: 102–120. doi:10.1016/j.isci.2019.01.006 [PubMed: 30682623]

39. Bludau I, Aebersold R. Proteomic and interactomic insights into the molecular basis of cell functional diversity. Nat Rev Mol Cell Biol. 2020;21: 327–340. doi:10.1038/s41580-020-0231-2 [PubMed: 32235894]

40. Huttlin EL, Bruckner RJ, Paulo JA, Cannon JR, Ting L, Baltier K, et al. Architecture of the human interactome defines protein communities and disease networks. Nature. 2017;545: 505–509. doi:10.1038/nature22366 [PubMed: 28514442]

41. Ikawa M, Wada I, Kominami K, Watanabe D, Toshimori K, Nishimune Y, et al. The putative chaperone calmegin is required for sperm fertility. Nature. 1997;387: 607–611. doi:10.1038/42484 [PubMed: 9177349]

42. Itcho K, Oki K, Gomez-Sanchez CE, Gomez-Sanchez EP, Ohno H, Kobuke K, et al. Endoplasmic Reticulum Chaperone Calmegin Is Upregulated in Aldosterone-Producing Adenoma and Associates With Aldosterone Production. Hypertension. 2020;75: 492–499. doi:10.1161/HYPERTENSIONAHA.119.14062 [PubMed: 31865789]

43. Anelli T, Sitia R. Protein quality control in the early secretory pathway. EMBO J. 2008;27: 315–327. doi:10.1038/sj.emboj.7601974 [PubMed: 18216874]

44. Pearl LH, Prodromou C. Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu Rev Biochem. 2006;75: 271–294. doi:10.1146/annurev.biochem.75.103004.142738 [PubMed: 16756493]

45. Bonifacino JS, Glick BS. The mechanisms of vesicle budding and fusion. Cell. 2004;116: 153–166. doi:10.1016/s0092-8674(03)01079-1 [PubMed: 14744428]

46. Kim DI, Roux KJ. Filling the Void: Proximity-Based Labeling of Proteins in Living Cells. Trends Cell Biol. 2016;26: 804–817. doi:10.1016/j.tcb.2016.09.004 [PubMed: 27667171]

47**. Samoudi M, Kuo CC, Robinson CM, Shams-Ud-Doha K, Schinn SM, Kol S, et al. In situ detection of protein interactions for recombinant therapeutic enzymes. Biotechnol Bioeng. 2021;118: 890–904. doi:10.1002/bit.27621 [PubMed: 33169829] Proximity-dependent biotin identification (BioID) method employed to label components of the secretory pathway interacting with different types of recombinant proteins containing different structural modifications. Applied Bayesian statistical modeling revealed specific machinery involved in disulfide bond formation, and N-glycosylation. Subsequent knock-down of measured interactor PDIA4 impacted secretion of disulfide bond dependent proteins, evaluating the potential of this methodology to identify targets for cell engineering.

48. Komatsu K, Kumon K, Arita M, Onitsuka M, Omasa T, Yohda M. Effect of the disulfide isomerase PDIa4 on the antibody production of Chinese hamster ovary cells. J Biosci Bioeng. 2020;130: 637–643. doi:10.1016/j.jbiosc.2020.08.001 [PubMed: 32878739]

49. Hefzi H, Ang KS, Hanscho M, Bordbar A, Ruckerbauer D, Lakshmanan M, et al. A Consensus Genome-scale Reconstruction of Chinese Hamster Ovary Cell Metabolism. Cell Syst. 2016;3: 434–443.e8. doi:10.1016/j.cels.2016.10.020 [PubMed: 27883890]

50. Calmels C, McCann A, Malphettes L, Andersen MR. Application of a curated genome-scale metabolic model of CHO DG44 to an industrial fed-batch process. Metab Eng. 2019;51: 9–19. doi:10.1016/j.ymben.2018.09.009 [PubMed: 30227251]

51**. Fouladiha H, Marashi S-A, Li S, Li Z, Masson HO, Vaziri B, et al. Systematically gap-filling the genome-scale metabolic model of CHO cells. Biotechnol Lett. 2021;43: 73–87. doi:10.1007/s10529-020-03021-w [PubMed: 33040240] This paper presents an updated genome-scale metabolic network model of CHO cells in which the number of reactions and pathways capable of carrying flux are substantially increased.

52. Yeo HC, Hong J, Lakshmanan M, Lee D-Y. Enzyme capacity-based genome scale modelling of CHO cells. Metab Eng. 2020;60: 138–147. doi:10.1016/j.ymben.2020.04.005 [PubMed: 32330653]

53. Li S, Richelle A, Lewis NE. Enhancing product and bioprocess attributes using genome-scale models of CHO metabolism. Cell Culture Engineering. Wiley; 2019. pp. 73–95. doi:10.1002/9783527811410.ch4

54*. Yusufi FNK, Lakshmanan M, Ho YS, Loo BLW, Ariyaratne P, Yang Y, et al. Mammalian Systems Biotechnology Reveals Global Cellular Adaptations in a Recombinant CHO Cell Line. Cell Syst. 2017;4: 530–542.e6. doi:10.1016/j.cels.2017.04.009 [PubMed: 28544881] The authors performed an integrative analysis combining multi-omics data and genome scale models to study the genotypic and phenotypic differences between a wild-type and recombinant antibody-producing Chinese hamster ovary (CHO) cell line.

55. Huang Z, Yoon S. Identifying metabolic features and engineering targets for productivity improvement in CHO cells by integrated transcriptomics and genome-scale metabolic model. Biochem Eng J. 2020;159: 107624. doi:10.1016/j.bej.2020.107624

56. Calmels C, Arnoult S, Ben Yahia B, Malphettes L, Andersen MR. Application of a genome-scale model in tandem with enzyme assays for identification of metabolic signatures of high and low CHO cell producers. Metab Eng Commun. 2019;9: e00097. doi:10.1016/j.mec.2019.e00097 [PubMed: 31720213]

57**. Gutierrez JM, Feizi A, Li S, Kallehauge TB, Hefzi H, Grav LM, et al. Genome-scale reconstructions of the mammalian secretory pathway predict metabolic costs and limitations of protein secretion. Nat Commun. 2020;11: 68. doi:10.1038/s41467-019-13867-y [PubMed: 31896772] *The authors integrated the mammalian secretory pathway with genome-scale reconstruction of human, mouse and CHO cells*, enabling the prediction of metabolic costs associated with protein secretion.

58. Schinn S-M, Morrison C, Wei W, Zhang L, Lewis NE. Systematic evaluation of parameters for genome-scale metabolic models of cultured mammalian cells. Metab Eng. 2021;66: 21–30. doi:10.1016/j.ymben.2021.03.013 [PubMed: 33771719]

59. Kol S, Ley D, Wulff T, Decker M, Arnsdorf J, Gutierrez JM, et al. Multiplex secretome engineering enhances recombinant protein production and purity. Nature Communications. doi:10.1101/647214

60. Nielsen J, Keasling JD. Engineering Cellular Metabolism. Cell. 2016;164: 1185–1197. doi:10.1016/j.cell.2016.02.004 [PubMed: 26967285]

61. O'Brien EJ, Monk JM, Palsson BO. Using Genome-scale Models to Predict Biological Capabilities. Cell. 2015;161: 971–987. doi:10.1016/j.cell.2015.05.019 [PubMed: 26000478]

62. Zhang J, Petersen SD, Radivojevic T, Ramirez A, Pérez-Manríquez A, Abeliuk E, et al. Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism. Nat Commun. 2020;11: 4880. doi:10.1038/s41467-020-17910-1 [PubMed: 32978375]

63. Sha, Sha S, Huang Z, Wang Z, Yoon S. Mechanistic modeling and applications for CHO cell culture development and production. Current Opinion in Chemical Engineering. 2018. pp. 54–61. doi:10.1016/j.coche.2018.08.010

64. Antonakoudis A, Barbosa R, Kotidis P, Kontoravdi C. The era of big data: Genome-scale modelling meets machine learning. Comput Struct Biotechnol J. 2020;18: 3287–3300. doi:10.1016/j.csbj.2020.10.011 [PubMed: 33240470]

65. Brunner M, Kolb K, Keitel A, Stiefel F, Wucherpfennig T, Bechmann J, et al. Application of Metabolic Modeling for Targeted Optimization of High Seeding Density Processes. Biotechnol Bioeng. 2021. doi:10.1002/bit.27693

66. Schinn S-M, Morrison C, Wei W, Zhang L, Lewis NE. A genome-scale metabolic network model and machine learning predict amino acid concentrations in Chinese Hamster Ovary cell cultures. Biotechnol Bioeng. 2021;118: 2118–2123. doi:10.1002/bit.27714 [PubMed: 33580712]

67. Kuo C-C, Chiang AW, Shamie I, Samoudi M, Gutierrez JM, Lewis NE. The emerging role of systems biology for engineering protein production in CHO cells. Curr Opin Biotechnol. 2018;51. doi: 10.1016/j.copbio.2017.11.015

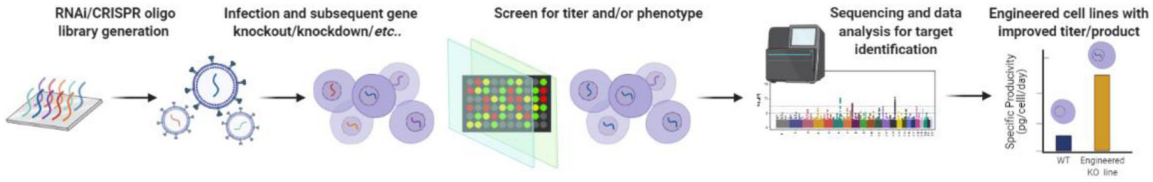

Figure 1.

Genetic screens using RNAi or CRISPR-Cas9 systems provide a high-throughput tool for gene target identification to boost protein production. While this method can help find engineering targets, it does not rely on understanding the underlying molecular mechanisms of the identified target. This can impede replicability or the ability to apply this engineering target in other contexts.

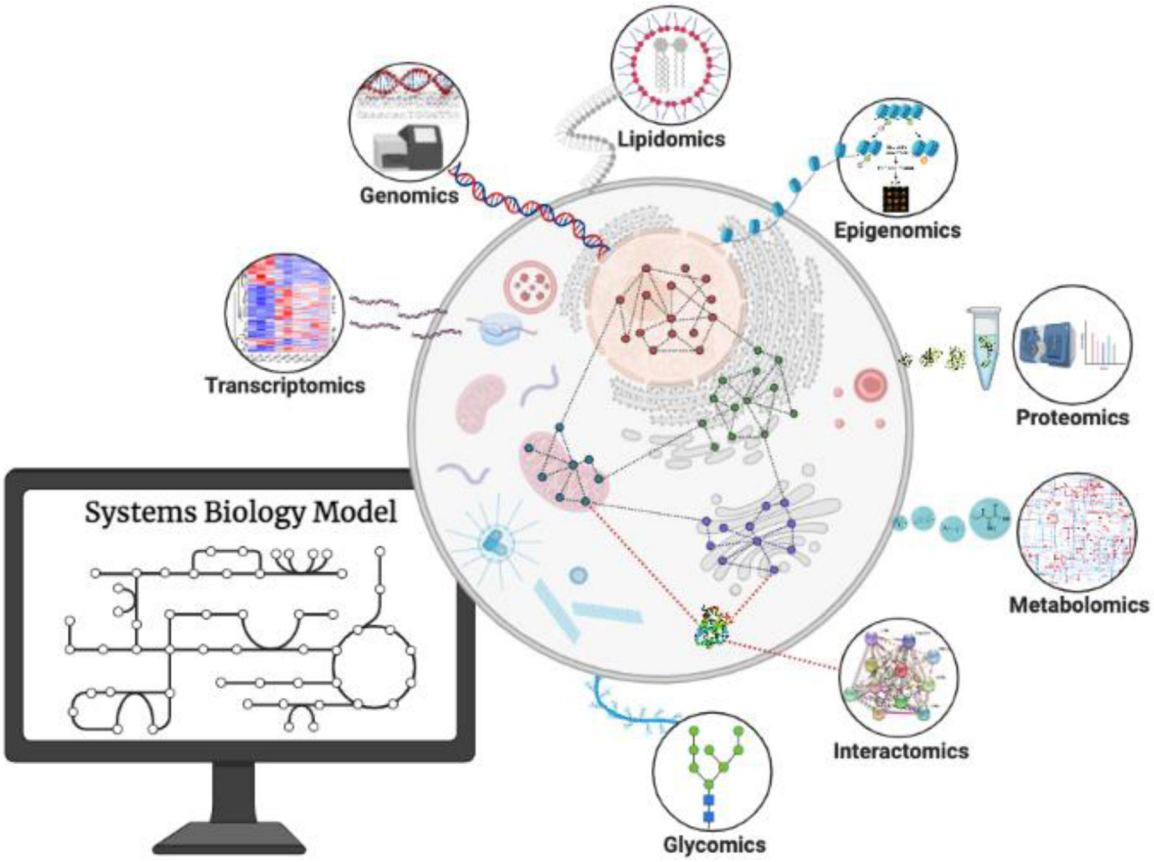

Figure 2.

Due to the importance of biologics in the healthcare field, there is increasing pressure in industry to efficiently develop high yield and robust cell lines for the manufacturing of therapeutics. The intersection of omics technologies and systems biology methods provides a unique opportunity to enhance our understanding of the mechanisms driving protein yield in cell culture, promoting a shift from empirical to rational approaches for cell line engineering.