Korean J. Chem. Eng., 39(5), 1097-1106 (2022)
DOI: 10.1007/s11814-022-1062-y
pISSN: 0256-1115
eISSN: 1975-7220

INVITED REVIEW PAPER

Energy metabolism in Chinese hamster ovary (CHO) cells: Productivity and beyond

Jong Uk Park‡, Hye-Jin Han‡, and Jong Youn Baik†

Department of Biological Sciences and Bioengineering, Inha University, 100, Inha-ro, Michuhol-gu, Incheon 22212, Korea
(*Received 12 October 2021 • Revised 6 January 2022 • Accepted 10 January 2022*)

Abstract−Chinese hamster ovary (CHO) cell lines have been widely used to produce recombinant proteins. While the biosynthesis of recombinant proteins is energy-intensive, CHO cells exhibit inefficient metabolism, characterized by rapid conversion of glucose to lactate, possibly leading to lower cell growth and productivity of therapeutic proteins. Therefore, it is important to understand and engineer cellular metabolism to increase recombinant protein production. In this review, cellular energy metabolism of CHO cells with respect to protein synthesis is overviewed. Then, genetic and process engineering approaches to enhance metabolic efficiency are described, resulting in the improvement of cell culture performance. Finally, recent modeling technologies for understanding and predicting cellular metabolic behaviors are reviewed. These efforts will aid to further advance the biomanufacturing of therapeutic proteins.

Keywords: Chinese Hamster Ovary (CHO) Cells, Biomanufacturing, Recombinant Protein, Energy Metabolism, Lactate Metabolic Shift

INTRODUCTION

Chinese hamster ovary (CHO) cells have been popular mammalian host cells for the production of various therapeutic proteins, such as monoclonal antibodies (mAbs), cytokines, and hormones [1-3]. CHO cells have several desirable characteristics suitable for biomanufacturing, including robust cell growth, adaptability to various culture environments, easy genetic manipulation, human-like post-translational modification, and being safe from human virus infection [3-5]. One hurdle to overcome is relatively low productivity compared to prokaryotic protein expression system [6]. For the last four decades, there have been many studies to improve productivity (i.e., protein production) and quality of glycoproteins in CHO cell culture [7,8]. These efforts include both cell engineering approaches, such as genetic modification and vector engineering, and bioprocess engineering approaches, such as culture parameter optimization and media development [9-12].

The biosynthesis of therapeutic proteins, or any proteins, involves two elements: materials (i.e., building blocks) and energy. Building blocks, such as amino acids for peptides and sugar nucleotides for glycans, can be either directly acquired from culture media or synthesized in the cells through anabolic pathways. Cellular energy, represented by adenosine triphosphate (ATP), is generated by various catabolic pathways that will be described later in this review. While building blocks are better recognized and investigated by many studies such as mass balance analyses and the development of chemically defined medium [13-15], the energy metabolism of CHO cells remains less clear in various aspects.

In this review, we first describe the current understanding about the metabolic pathways of energy production and consumption in CHO cells. Then, we survey cell and process engineering approaches to improve energy metabolism. Finally, we update recent modeling approaches to understand and regulate complex cellular metabolism.

ENERGY METABOLISM OF CHO CELLS

Like other mammalian cells, CHO cells mainly utilize glucose to produce ATP via either glycolysis in cytosol or the tricarboxylic acid (TCA) cycle followed by oxidative phosphorylation (OXPHOS) in mitochondria. And the cellular energy generated from nutrients is used in various metabolic activities such as proliferation, maintenance, protein synthesis, and nucleotide synthesis (Fig. 1). While the TCA cycle and OXPHOS produce much more ATP than glycolysis (36 ATP vs. 2 ATP per one glucose), the metabolic characteristics of CHO cells exhibit high glycolytic flux with high glucose consumption and lactate production rates [16,17]. This high glycolytic flux not only inefficiently produces ATP but also generates excessive lactate that causes acidification of media, resulting in an increase in osmolality in bioreactor cultures due to the base addition as a pH control. Hyperosmolality has negative effects on culture parameters, such as cell proliferation, productivity, and product quality [18-20].

1. Rapid Lactate Accumulation During the Exponential Growth Phase

The glucose metabolism of CHO cells in the early exponential growth phase is characterized by high glucose uptake rate as well as high lactate production rate. This feature has also been found in other fast-growing cells such as cancer cells, mammalian cell lines, and even yeasts, and is termed as Warburg effect (or aerobic glycolysis) [21-23]. To date, several hypotheses have been proposed to explain this high glycolytic flux. The first hypothesis is that the primary purpose of aerobic glycolysis in fast-growing cells is just to support rapid cell proliferation. It was originally suggested that cells need to utilize aerobic glycolysis to quickly meet the require-

†To whom correspondence should be addressed.
E-mail: jybaik@inha.ac.kr
‡These authors contributed equally to the work described in this paper.

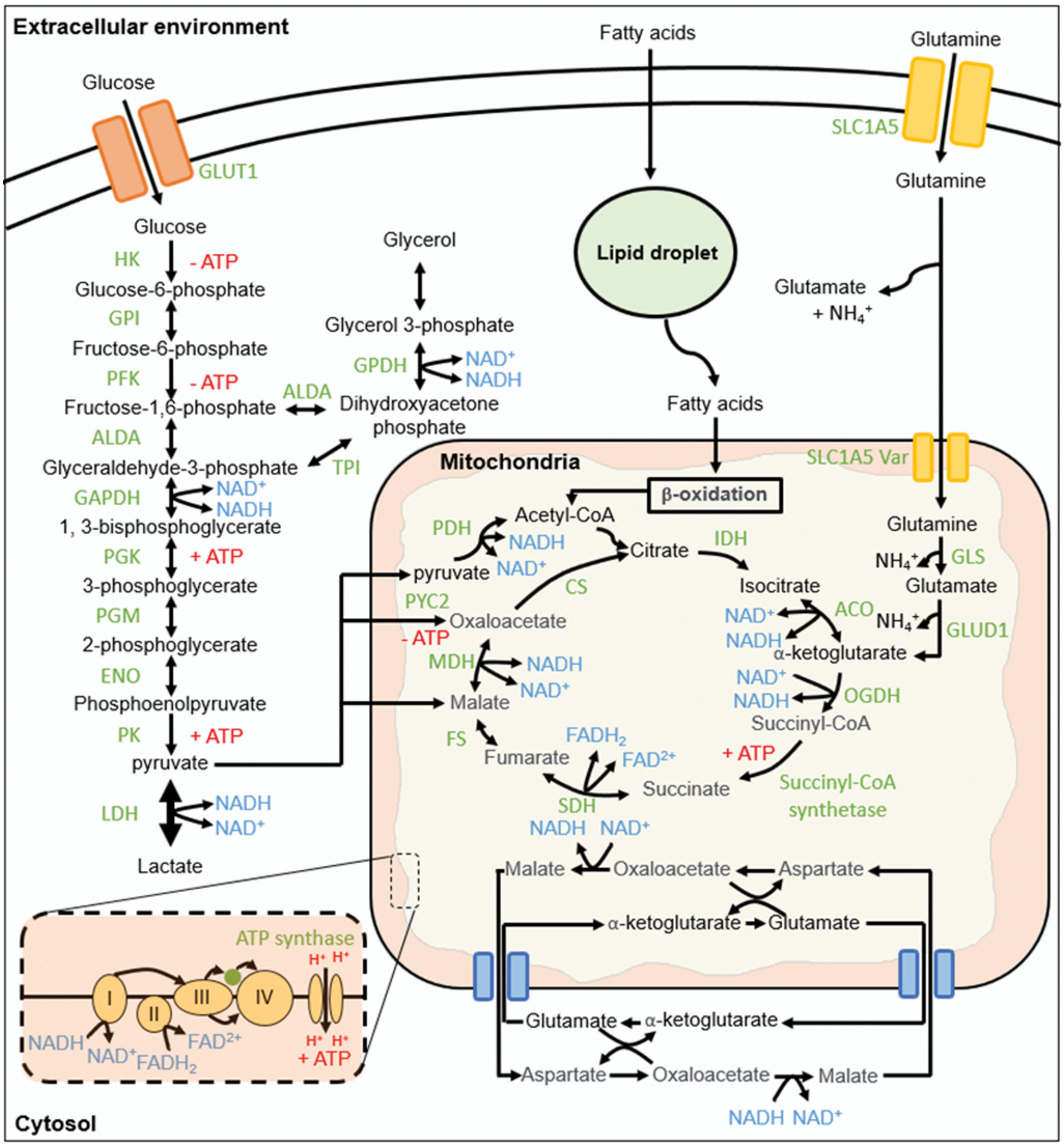

Fig. 1. A schematic diagram of energy generation metabolic pathways. Green, blue, and red color fonts indicate enzymes, NAD or FAD cofactors, and ATP production/consumption, respectively. ACO; aconitase, ALDA; aldolase A, CS; citrate synthase, ENO; enolase, FS; fumarase, GAPDH; glyceraldehyde 3-phosphate dehydrogenase, GLS; glutaminase, GLUD1; glutamate dehydrogenase 1, GLUT; glucose transporter, GPDH; glycerol-3-phosphate dehydrogenase, GPI; glucose 6-phosphate isomerase, HK; hexokinase, IDH; isocitrate dehydrogenase, LDH; lactate dehydrogenase, MDH; malate dehydrogenase, OGDH; oxoglutarate dehydrogenase, PDH; pyruvate dehydrogenase, PFK; phosphofructokinase, PGK; phosphoglycerate kinase, PGM; phosphoglycerate mutase, PK; pyruvate kinase, PYC2; pyruvate carboxylase 2, SLC1A5; solute carrier family 1 member 5, SDH; succinate dehydrogenase, TPI; triosephosphate isomerase.

ments of building blocks and energy for rapid growth; glycolysis generates ATP 10-100 times faster than OXPHOS [21]. Indeed, Martinez et al. reported that approximately 45% of total ATP was generated by aerobic glycolysis during the exponential phase of CHO cells, and that 58% of total ATP produced during the glucose consumption phase was utilized in biomass production, such as protein, fatty acid, nucleotide, and polysaccharide synthesis [15]. However, several studies reported that an average of 17% of total ATP is produced via glycolysis in 31 types of cancer cells (or tissues) and that synthesis of building blocks accounted for only ~7% of glucose uptake, whereas the remaining 93% of glucose uptake was used for by-product generation such as lactate and alanine [24-26]. Furthermore, it was reported that most of the ATP was produced via OXPHOS in other various cancer cells and primary cells [27], suggesting that the amount of ATP required for cell proliferation may be much less than that required for cellular maintenance [15,21]. The discrepancy of energy metabolism between these different cell types has not been elucidated in detail. The second hypothesis is that aerobic glycolysis restores the nicotinamide adenine dinucleotide $(NAD)^+$/NADH redox balance by producing NAD^+ through lactate production. NAD is an essential coenzyme that transfers electrons in various metabolic pathways such as cellular redox state maintenance and energy metabolism [8]. Two molecules of NAD^+ are converted to two NADH via glycolysis of one glucose mole-

cule. Under a normal physiological glucose condition (less than 10 mM blood glucose level), this NAD^+/NADH redox state is maintained at a consistent level by the malate-aspartate shuttle in mitochondria that recycles cytosolic NADH into NAD^+ [28]. However, much higher glucose concentration in culture media (typically 30 mM) would lead to higher glucose uptake, glycolytic flux, and NADH level (from the conversion of glyceraldehyde to 1, 3-bisphosphoglycerate mediated by GAPDH), which would exceed the NAD^+/NADH homeostasis capacity of malate-aspartate shuttle [28]. To balance the NAD^+/NADH ratio, cells may convert excessive pyruvate into lactate using lactate dehydrogenase (LDH) and NADH, producing NAD^+ [28].

Lactate accumulation can adversely affect both small- and large-scale cell cultures. While small-scale cultures are usually conducted in shake flasks or multi-well plates with minimal means of parameter control (temperature, humidity, and shaking speed), large-scale cultures typically involve the use of bioreactors with much more controls (temperature, pH, agitation speed, dissolved oxygen, foam, level, etc.). Assuming a typical amount of lactate accumulation (1 g/L/day) during the exponential growth phase [15,20], we can estimate the number of protons from lactate dissociation (the pKa of lactate is 3.86 at 25 °C) as well as the amount of NaOH that should be added to neutralize it. The calculation suggests that more than 1.26×10^{-3} M protons are added daily in the culture medium, which would drop the medium pH from 7.2 to 2.7 in three days if no pH buffering system is considered. In large-scale cultures, medium pH is controlled with acid (CO_2 gas) and base (sodium hydroxide or sodium bicarbonate). When 1 g/L/day of lactate is accumulated, 11.1 mM sodium hydroxide should be added to maintain the medium pH, and the resulting salt (i.e., sodium lactate) would increase the medium osmolality daily by 22.2 mOsm/kg. These changes in medium pH or osmolality negatively impact cell growth, productivity, and/or product quality [18-20].

2. Metabolic Shift from Lactate Production to Consumption

Metabolic profiles are an important parameter for cell line screening in the CHO cell line development. Clones suitable for industrial production are selected by comparing various culture parameters such as growth, viability, and titer. During this process, it is observed that a number of clones show a phenotype that shifts from lactate production to the lactate consumption with the activation of the TCA cycle and OXPHOS [20]. This phenotype, called the lactate metabolic shift, seems to be promoted when glucose or glutamine level is low in the late-exponential or stationary phase [29-32]. While the mechanism that triggers the lactate metabolic shift is still unclear, there is a good positive correlation between this phenotype and high glycoprotein productivity [20,33-35].

Accumulating evidence suggests that lactate consumption is related to a higher energy metabolic state. Zagari et al. investigated the carbon metabolism in parental CHO-S cell lines with the lactate metabolic shift phenotype as well as subclones that produce excessive lactate throughout the cultures [29]. The subclones with excessive lactate accumulation exhibited a decrease in both mitochondrial membrane potential and oxygen consumption rate from the exponential phase compared to the parental CHO-S cells, suggesting that the lactate accumulation in these subclones is related to the impaired mitochondrial oxidative capacity. Furthermore, Jing et al. treated CHO cells with the inhibitors of glycolysis and OXPHOS, respectively, and observed that ATP in the endoplasmic reticulum (ER) came from mitochondria through a cytosolic Ca^{2+}-antagonized ATP transport [36]. Given that protein folding, post-translational modification, trafficking, and secretion processes take place in the ER, a substantial amount of energy is required in the ER for the recombinant protein production. Therefore, the cells that uptake (i.e., consume) lactate from media, that convert lactate into pyruvate and further into acetyl-CoA, and that use acetyl-CoA to fuel the TCA cycle would meet higher energy demand in the ER, thereby becoming higher producers [35-37].

3. Amino Acid and Lipid Metabolism in CHO Cells

CHO cells can also generate energy from other nutrients than glucose. Via glutaminolysis, cytosolic glutamine is uptaken into mitochondria [38,39] and converted to glutamate by glutaminase, then to α-ketoglutarate (α-KG), a TCA cycle intermediate, by glutamate dehydrogenase. It was reported that 32% of ATP was derived from glutamine in CHO cells and that there was a correlation between the lactate metabolic shift (consumption) and glutamine depletion in the culture media [15]. It is hypothesized that α-KG from glutaminolysis saturates the TCA cycle and reduces the influx of pyruvate, delaying lactate consumption until glutamine is depleted [15,30,32].

Fatty acids such as linoleic acid and myristic acid can function as a long-term energy storage by forming lipid droplets in cells [40,41]. When the intracellular energy source is limited, CHO cells can produce acetyl-CoA via the β-oxidation of fatty acids to fuel the TCA cycle in mitochondria [42]. While lipid components such as ethanolamine, choline, and fatty acids are included in serum-free chemically-defined culture media for this reason [41], lipid metabolism has received little attention so far.

4. Energy Usage in CHO Cells

To determine if cellular energy is a limiting factor for recombinant protein production, it is necessary to assess how much energy is used and/or required for protein synthesis. Martinez et al. employed flux balance analysis (FBA) in the CHO-XL99 cell line to determine energy generated and used in CHO cells and observed that 45% and 35% of total cellular ATP was generated from glucose and glutamine, respectively [15]. In addition, a substantial amount of energy, 39% of the total ATP, was used for protein synthesis with sufficient energy sources, suggesting that energy limitation can be a contributing factor for difficult-to-express (DTE) protein issues. While causes and bottlenecks of DTE protein production remain largely unclear, a novel approach by Gutierrez et al. suggested cellular energy as a limiting factor for DTE protein synthesis [43]. In this study, energy requirements for the biosynthesis and secretion of endogenous CHO cell proteins and recombinant proteins were estimated using CHO proteomic data and stoichiometric consideration [43-46]. For example, domains (e.g., signal peptides), glycosyl phosphatidylinositol anchor attachment, and the number of N-linked and/or O-linked glycosylation, were identified, followed by the calculation of energy cost for charging and polymerizing amino acids into peptides, protein folding, and vesicular transport [44-47]. Based on these energy calculations of representative biopharmaceuticals produced in CHO cells, Factor VIII, a well-known DTE protein, exhibited much higher energy

requirement (9,488 ATP) compared to other CHO endogenous proteins (generally under 4,000 ATP) [43]. These results suggest that increasing the energy supply to protein synthesis pathways can be an effective strategy to improve the productivity of the DTE or any proteins.

ENGINEERING APPROACHES TO IMPROVE CHO CELL METABOLISM

Several strategies employed to improve the energy metabolism of CHO cells for higher productivity can be grouped into two categories: cell engineering and process engineering. As cell engineering approaches involve genetic modification of cell lines, these studies generally exhibit more stable and clearer effects of modification but are time-consuming [48,49]. Furthermore, genetic modification of existing commercial cell lines is not directly applicable due to regulatory issues. Process engineering involves the optimization of "environmental" factors, such as culture parameters, media components, additives, and feeding strategies. Process engineering approaches are responsive, easier to handle, and can be high throughput, but results may seem less obvious [41,50].

1. Cell Engineering Strategies

LDH activity has been the primary target to reduce lactate accumulation or to induce the lactate metabolic shift. Cytosolic pyruvate produced via glycolysis is oxidized by LDH into lactate. Among the isotypes of LDH, *ldhA* and *ldhB* are mainly expressed in CHO cells [8]. *ldhA* encodes an LDH-M protein having a higher affinity for pyruvate whereas *ldhB* encodes an LDH-H protein having a higher affinity for lactate [8]. Lee et al. tested the effect of suppressing *ldhA* expression in CHO cells and reported that when *ldhA* was inhibited, lactate production reduced and specific productivity increased [51]. In addition, they overexpressed *bcl-2*, an anti-apoptotic gene, and silenced *ldhA* using a siRNA in the CHO cells producing Fc-fusion protein, and observed a decrease in lactate accumulation along with even higher recombinant protein productivity compared to when *ldhA* alone was silenced [52]. The authors also argued that the flux increase in the TCA cycle and OXPHOS due to *ldhA* suppression might lead to apoptosis by increasing ROS levels in these cells [52,53].

Overexpression of pyruvate carboxylase 2 (PYC2) is another strategy to enhance the lactate metabolic shift by rewiring the metabolic flux from glycolysis to the TCA cycle and OXPHOS. PYC converts pyruvate to oxaloacetate, a TCA cycle intermediate, thereby fueling the TCA cycle while reducing pyruvate and lactate accumulation. Because endogenous PYC may not sufficiently convert accumulated pyruvate from the high glycolytic flux, *PYC2* derived from yeasts was overexpressed in several mammalian cell lines [54-58]. The *PYC2*-positive clones showed lower glucose consumption and lactate production than the *PYC2*-negative clones and parental cells [56]. Along with the higher peak cell density and comparable viability, the *PYC2*-positive clones exhibited a 35% increase in productivity compared to the non-engineered cells [56].

2. Process Engineering Strategies

2-1. Media Components and Additives

Alternative sugars, such as fructose, maltose, and sucrose, were tested as a strategy to reduce excessive glucose uptake and resulting lactate accumulation. For example, Wlaschin and Hu replaced glucose with fructose and also overexpressed *slc2a5*, a fructose transporter, leading to a reduction in lactate accumulation while maintaining a comparable cell growth [59]. In addition, Ng et al. tested maltose-, sucrose-, lactose- and trehalose-containing media to adapt three cell lines for 72 days, and found that the maltose-treated cultures exhibited comparable cell density and viability but significantly slower growth rate (doubling time: 53.7 hour with maltose medium vs. 22.3 hour with glucose medium) [60,61]. These results suggest that maltose can be used in a biphasic manner together with glucose; glucose is first consumed at the beginning of the culture (day 0-4) and maltose is consumed thereafter [60]. Supplementing galactose can also reduce lactate accumulation by adjusting glucose metabolism [62]. Torres et al. fed galactose and lactate in the culture medium upon glucose depletion and observed that the consumption of both galactose and amino acids decreased, resulting in more sustained cell growth and protein production [63].

Media additives that regulate the expression and activity of enzymes involved in metabolic pathways have also been tested to modulate the efficiency of energy metabolism [64-66]. For example, pyruvate dehydrogenase complex is a key enzyme complex that converts pyruvate to acetyl-CoA, and its activity can be inhibited by the phosphorylation of Ser232, Ser293, and Ser300 mediated by pyruvate dehydrogenase kinase (PDK) [64,65]. Buchsteiner et al. tested dichloroacetate, a PDK inhibitor, on multiple batch and fed batch cultures of antibody-expressing cells and observed a 35% and 40% decrease in glucose uptake and lactate production, respectively [64]. Another example is the treatment of resveratrol, an activator of Sirtuin 1, to CHO cell cultures [66]. Sirtuin 1, an NAD-dependent deacetylase, regulates the activity of several enzymes involved in cellular metabolism, and enhances mitochondrial OXPHOS [67]. The resveratrol-treated CHO cultures exhibited an increase in specific productivity in a dose-dependent manner [66].

2-2. Culture Process Parameters

pH seems a critical culture process parameter to control the lactate metabolic shift during cell culture processes [8,68-71]. Ivarsson et al. investigated the relationship between pH and lactate metabolism in a murine hybridoma cell line by varying the culture pH at the early exponential phase [10]. In the low pH culture (pH 6.8), both glucose uptake and lactate production decreased compared to the other conditions (pH 7.2 and pH 7.8), suggesting that lactate uptake mediated by monocarboxylate transporter is more active at lower pH, thereby increasing the lactate influx as well as OXPHOS [8]. Partial carbon dioxide pressure or pCO_2, a culture parameter involved in the pH buffering system, can also affect lactate metabolism in a pH-independent manner. Brunner et al. tested various levels of pCO_2 and observed that high pCO_2 inhibited the lactate metabolic shift regardless of the culture pH [71].

The relationship between the lactate metabolic shift and media pH can suggest a novel culture process control strategy [72,73]. (1) At the beginning of a fed-batch culture, lactate gets accumulated via glycolysis, lowering pH. (2) When glucose level is low, the lactate metabolic shift occurs (lactate consumption), leading to higher pH. (3) At a certain pH setpoint (high-end), when the both glucose and lactate levels are low, a small amount of glucose is fed to the culture and lactate accumulation reoccurs [72]. This high-end

pH delivery of glucose (HIPDOG) strategy, which considers the culture pH as a sensor of the metabolic state in the cells, has several advantages; it reduces the accumulation of glucose and lactate, and the medium osmolality does not substantially change as no base is added. The application of HIPDOG technology in CHO fed-batch cultures achieved a dramatically higher cell density and thereby increased the recombinant protein production over 4.1-fold than the control culture [73].

APPLICATION OF METABOLIC MODEL FOR THE PRODUCTION OF THERAPEUTIC PROTEINS

Although high-yield processes have been achieved through many cell line and process development studies, these efforts are usually empirical and may involve cost- and time-consuming steps [74]. For more effective engineering of CHO cell cultures, it is desirable to understand and regulate the behavior of CHO cells. With the development of omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics, mathematical models that predict CHO cellular metabolism are becoming more accurate and sophisticated [75,76]. Many models used in CHO cell metabolism studies can be categorized into stoichiometric models and kinetic models, and their characteristics, development processes, and applications are summarized in Table 1, Table 2, and Fig. 2 (for a more detailed review of these mathematical models, please refer to [74-83]. Stoichiometric models, such as metabolic flux analysis (MFA), FBA, and genome-scale metabolic models (GEMs), are applied to estimate intracellular fluxes [79]. While even larger network models (i.e., GEMs) can be constructed with the limited information on individual reactions, calculating metabolite concentrations and simulating with time changes can be inaccurate. These stoichiometric models can be substantially improved with relevant

Table 1. Characteristics of stoichiometric models and kinetic models [79,96,97]

	Stoichiometric model	Kinetic model
Model size	Large, genome-scale models	Small models
Model parameters	Not required	Requires kinetic parameters
Constraints	Mass balance Energy balance Thermodynamics Enzyme capacity	Stability of steady state Duration of transition process
Description	Quantitative flux calculation Static description	Dynamic cell metabolism Detailed quantitative description Prediction of quantitative flux or concentration
Types of models	MFA, FBA, GEM	Monod kinetics, mass action kinetics, Michaelis-Menten kinetics

FBA; flux balance analysis, MFA; metabolic flux analysis, GEM; genome-scale metabolic mode

Table 2. Applications of *in silico* metabolic models in mammalian cell cultures processes

Study	Cell line	Approach	Experimental /Input data	Prediction /Output data	Summary	Reference
Altamirano et al.	CHO	Stoichiometric model	Specific consumption/ production rates for major metabolism, specific cell growth rate, average cell composition	Intracellular fluxes of the reactions	Analysis of the metabolic dynamics of CHO cells with the perspective of glucose and galactose, and lactate metabolism through metabolic flux analysis	[84]
Sheikholeslami et al.	CHO	Metabolic network model	Simplified reactors for the synthesis of biomass and antibody	Key intracellular flux distributions correlated with either cell growth or productivity	Evaluation of the effects of glutamine feeding on cell metabolism and recombinant protein production and establishment of glutamine feeding strategy through ^{13}C-metabolic flux analysis	[85]
Ben Yahiaet al.	CHO	Monod-inhibition type kinetics model	The maximum concentration of cysteine and tryptophan on day 3, and the specific productivity on day 3	The specific growth rate, mAb titer, metabolites concentration	Prediction of the impact of cysteine and tryptophan on cell growth, metabolism and mAb production and optimization of feeding conditions in fed-batch culture	[87]

GEM; Genome-scale metabolic model, PSIM; Protein-specific information matrix, VCD; Viable cell density

Table 2. Continued

Study	Cell line	Approach	Experimental /Input data	Prediction /Output data	Summary	Reference
Selvarasu et al.	Murine hybridoma	Multivariate statistical data analysis	Specific rates of consumption/production (specific growth rate, specific substrate consumption rate, specific production rate)	Cell growth, antibody production	Evaluation of the optimal concentrations of key amino acids in feed medium, increase in cell viability and productivity, and decrease in toxic waste production through multivariate statistical analysis	[88]
Calmels et al.	CHO	GEM	Daily experimental uptake/production rates of 24 metabolites	Cell growth, metabolite secretion and production rates, amino acid utilization	Improvement of the model by adding reaction related to high-yielding production cell metabolism and simplification of the existing model, application of it to industrial fed-batch production	[74]
Huang et al.	CHO	GEM	The uptake and secretion rates of metabolites (amino acids, glucose, lactate, ammonium)	Cell growth rates, specific IgG productivity	Development of a modeling-based approach for media optimization to increase IgG productivity based on transcriptomics data	[92]
Calmels et al.	CHO	GEM	Flux rates calculated from daily experimental uptake/production rates of 24 metabolites in medium	Specific cell growth rate, nutrient consumption rates, by-product production rates, and enzymatic activity	Characterization of high and low producers and identification of bottlenecks in a number of metabolic pathways	[93]
Huang et al.	CHO	GEM	Time-series gene expression profiles (RNA-Seq, day 3 to day 6), and extracellular metabolomics	Specific cell growth rate	Evaluation of time-series transcriptomic data on the robustness of genome-scale models	[94]
Gutierrez et al.	CHO	GEM	PSIM	Specific productivity of IgG	Reconstruction of GEM containing the secretory pathway of mammalian cells and calculation of the energy required for the protein production and improvement of specific productivity by regulating the gene expression level required lots of energy but not related to the production of recombinant proteins	[43]
Schmitt et al.	CHO	Machine learning	Concentration of metabolites and process data	Lactate profile at the late stage	Construction of model predictive controller by using cell culture process data and machine learning techniques and improvement of specific productivity via the suppression of the lactate accumulation in bioreactor	[90]

parameters or constraints, such as reaction directionalities and lower or upper boundaries [79,81]. Kinetic models are presented in a series of ordinary differential equations and have been used to explain dynamic changes, such as metabolite concentration, cell density, and recombinant protein production during the culture processes [79]. While these kinetic models provide more quantitative and accurate predictions with time changes, they require more experimental data for calibrations and also are prone to be heavily

Table 2. Continued

Study	Cell line	Approach	Experimental /Input data	Prediction /Output data	Summary	Reference
O'Brien et al.	CHO	Hybrid model	Experimental time course data for glucose, lactate, and osmolarity, starting concentrations of glucose and lactate	Glucose, lactate, VCD, and osmolarity	Description of the different metabolic phase in CHO cell culture and the variability in manufacturing runs by integrating a mechanistic metabolic model with subcomponent models for cell growth, signaling regulation, and the bioreactor environment	[86]
Schinn et al.	CHO	Hybrid model	Metabolomics measurements - (1) VCD and titer measurements (2) Bioreactor concentrations of glucose, lactate, glutamate, and glutamine)	Early amino acids consumption rates	Explanation of time-course dependent production of amino acid concentration by integrating machine learning with the CHO metabolic models	[91]
Robert J. Lovelett et al.	CHO	Hybrid model	Asparagine, glutamate, and copper level in media	mAb titer, VCD, total cell density, and metabolite concentrations	Development of a combined model to efficiently simulate the change of metabolism and glycosylation during cell culture	[95]

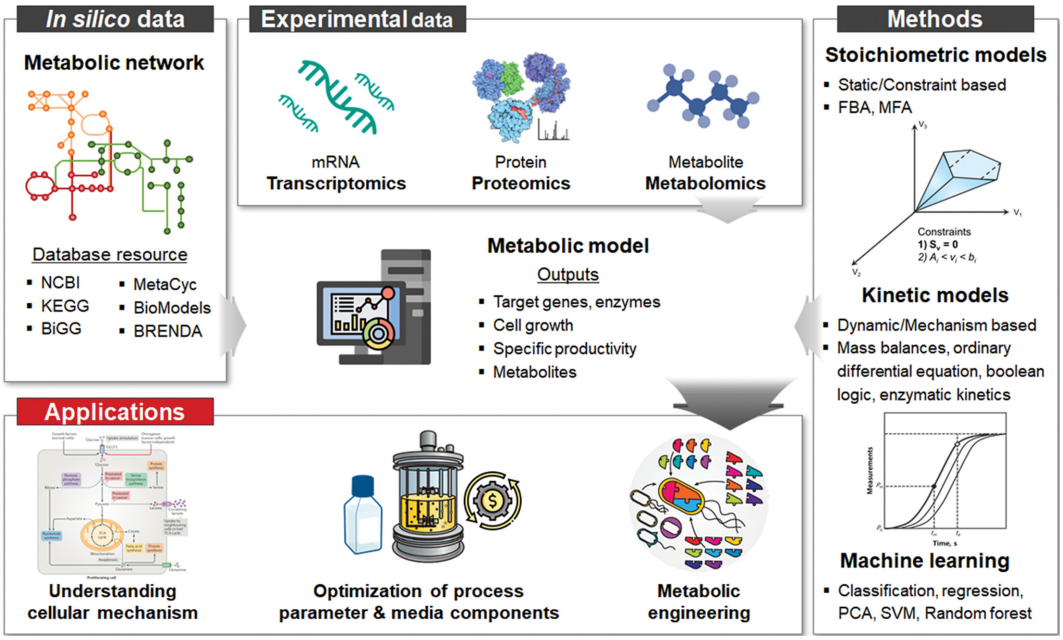

Fig. 2. Development process and applications of *in silico* metabolic model. To construct the genome-scale metabolic models (GEMs), genome annotation and metabolic databases are obtained from database resources such as NCBI and KEGG. Experimental information including transcriptomics, proteomics, and metabolomics could be helpful to develop more sophisticated models. Depending on the size of the networks and constraints, metabolic models can be classified into stoichiometric models and kinetic models; more recently, a machine learning technique has been applied to construct the GEMs. The developed models are used to understand intracellular metabolism, to optimize processes and cell culture media, and to perform metabolic engineering. FBA; flux balance analysis, MFA; metabolic flux analysis [76].

parameterized [79,81].

Two principles have been proposed for the construction of relevant models: (1) While empirical models are suitable for mass transfer and single substrate-limited processes, mechanistic models are preferred for interactive enzymes or multiple rate-limiting reactions. (2) Empirical models containing fewer kinetic parameters are

applicable for a fast-screening step of operation condition and a quick view of culture process properties. On the other hand, mechanistic models with more kinetic parameters are recommended for the optimization of cell culture parameters, providing specific metabolic mechanisms [78].

1. Understanding CHO Cell Metabolism and Cell Culture Processes

In silico metabolic models can help researchers to understand complex cellular metabolism and to obtain foundational knowledge for further bioprocess engineering. Altamirano et al. investigated the metabolic behavior of CHO cells using MFA and provided insights into lactate metabolism [84]. ^{13}C-MFA showed a substantial increase in the glycolytic flux as well as the amount of pyruvate (66% of total pyruvate) that entered the TCA cycle in the culture with a low glutamine feed, suggesting an effective feeding strategy for higher productivity [85]. In addition, as described in the Section 2.4., Gutierrez et al. integrated GEMs with the secretory pathway to calculate the energy cost of CHO endogenous proteins as well as recombinant proteins. Using this information, the CHO endogenous proteins with high energy cost were downregulated to improve the overall energy metabolism [43].

2. Process Parameter Optimization via Modeling

Modeling technologies can provide rationales to optimize culture process parameters that can achieve a high yield process and maintain the desired product quality [86]. Yahia et al. validated that the concentration of cysteine and tryptophan affected the cell growth and protein production. Then, they applied a Monod type equation (kinetic model) to the *in silico* prediction for optimal operating conditions [87]. In addition, Selvarasu et al. identified key amino acids in feed medium that can improve cell growth and mAb production using multivariate statistical analysis [88]. For example, glycine, tyrosine, phenylalanine, methionine, histidine, lysine, valine, isoleucine, and leucine were positively correlated with the higher mAb production showing improved membrane stability during the later growth phase. On the other hand, aspartate, glutamate, and alanine addition suppressed the cell growth and mAb production [88]. Serine and asparagine have a positive effect on cell growth, but a negative effect on protein production, suggesting that the concentration of these amino acids needs to be carefully determined in the media or feed [88].

3. Metabolic Models Combined with Machine-learning Approaches

Although computational modeling approaches are widely applied to analyze biomanufacturing processes, accurate simulation or prediction still remains a challenge due to the complexity of living cells. In this regard, applying machine learning (ML), which can automatically learn from the complex patterns and make intelligent decisions based on data, to the conventional metabolic models could provide an alternative approach for modeling complex biomanufacturing processes with incomplete understanding of CHO cell metabolism and biology [75,89]. Schmitt et al. predicted the lactate metabolic state at the late stage of cell culture by using a ML approach with the process data of early culture days [90]. Based on this model-predictive control that automatically manipulates pH setpoint as well as feed volume, a robust cell culture process with the improved lactate consuming state was successfully developed [90]. In addition, Schinn et al. integrated ML technology into a GEM to predict amino acid consumption rates during early culture days. Major limitations of conventional GEMs are that they are easily overfitted and that they may not be valid when the steady state assumptions are not applicable (e.g., changes in metabolic states) [91]. The ML-powered GEM mitigated these limitations and resulted in more accurate and time-course dependent prediction of amino acid concentration in the medium. However, obtaining and processing a large number of process datasets is a major obstacle of ML strategies.

CONCLUSION

To meet a continuously increasing demand for biopharmaceutical production, better understanding of cellular metabolic activities regarding recombinant protein synthesis pathway would provide effective bioprocessing strategies. In this regard and given that protein biosynthesis requires a large amount of cellular energy, represented by ATP, understanding and regulating energy-producing pathways in CHO cells may improve cellular metabolism, thereby increasing recombinant protein productivity. In this review, we discussed the current understanding of CHO energy metabolism and related engineering efforts to predict, control, and innovate culture processes. As our knowledge about CHO energy metabolism and its regulation is improving, we expect that there will be great progress towards effective and efficient biomanufacturing in the near future.

ACKNOWLEDGEMENT

This work was supported by INHA University Research Grant.

REFERENCES

1. G. Walsh, *Nat. Biotechnol.*, **36**, 1136 (2018).
2. A. L. Grilo and A. Mantalaris, *Trends Biotechnol.*, **37**, 9 (2019).
3. M. M. Zhu, M. Mollet, R. S. Hubert, Y. S. Kyung and G. G. Zhang, *Handbook of industrial chemistry and biotechnology*, 3rd edition, Springer (2017).
4. T. Lai, Y. Yang and S. K. Ng, *Pharmaceuticals (Basel)*, **6**, 579 (2013).
5. S. Pereira, H. F. Kildegaard and M. R. Andersen, *Biotechnol. J.*, **13**, e1700499 (2018).
6. G. L. Rosano and E. A. Ceccarelli, *Front. Microbiol.*, **5**, 172 (2014).
7. M. N. Henry, M. A. MacDonald, C. A. Orellana, P. P. Gray, M. Gillard, K. Baker, L. K. Nielsen, E. Marcellin, S. Mahler and V. S. Martínez, *Biotechnol. Bioeng.*, **117**, 1187 (2020).
8. F. Hartley, T. Walker, V. Chung and K. Morten, *Biotechnol. Bioeng.*, **115**, 1890 (2018).
9. J. Horvat, M. Narat and O. Spadiut, *Biotechnol. Prog.*, **36**, e3001 (2020).
10. M. Ivarsson, H. Noh, M. Morbidelli and M. Soos, *Biotechnol. Prog.*, **31**, 347 (2015).
11. J. J. Cacciatore, L. A. Chasin and E. F. Leonard, *Biotechnol. Adv.*, **28**, 673 (2010).
12. M. G. Gonzalez, Y. Latorre, R. Zuniga, J. C. Aguillon, M. C. Molina and C. Altamirano, *Crit. Rev. Biotechnol.*, **39**, 665 (2019).

13. E. J. M. Blondeel and M. G. Aucoin, *Biotechnol. Adv.*, **36**, 1505 (2018).
14. B. Beyer, M. Schuster, A. Jungbauer and N. Lingg, *Biotechnol. J.*, **13**, 1700476 (2018).
15. V. S. Martinez, S. Dietmair, L. E. Quek, M. P. Hodson, P. Gray and L. K. Nielsen, *Biotechnol. Bioeng.*, **110**, 660 (2013).
16. J. D. Young, *Curr. Opin. Biotechnol.*, **24**, 1108 (2013).
17. P. R. Rich, *Biochem. Soc. Trans.*, **31**, 1095 (2003).
18. N. Romanova, T. Niemann, J. F. W. Greiner, B. Kaltschmidt, C. Kaltschmidt and T. Noll, *Biotechnol. Bioeng.*, **118**, 2348 (2021).
19. S. Alhuthali, P. Kotidis and C. Kontoravdi, *Int. J. Mol. Sci.*, **22**, 3290 (2021).
20. P. L. Lieske, W. Wei, K. B. Crowe, B. Figueroa and L. Zhang, *Biotechnol. J.*, **15**, e1900306 (2020).
21. M. V. Liberti and J. W. Locasale, *Trends Biochem. Sci.*, **41**, 211 (2016).
22. M. G. Vander Heiden, L. C. Cantley and C. B. Thompson, *Science*, **324**, 1029 (2009).
23. O. Warburg, *Cancer Res.*, **9**, 148 (1925).
24. D. A. Hume, J. L. Radik, E. Ferber and M. J. Weidemann, *Biochem. J.*, **174**, 703 (1978).
25. R. J. DeBerardinis, A. Mancuso, E. Daikhin, I. Nissim, M. Yudkoff, S. Wehrli and C. B. Thompson, *Proc. Natl. Acad. Sci.*, **104**, 19345 (2007).
26. S. Y. Lunt and M. G. Vander Heiden, *Annu. Rev. Cell Dev. Biol.*, **27**, 441 (2011).
27. X. L. Zu and M. Guppy, *Biochem. Biophys. Res. Commun.*, **313**, 459 (2004).
28. J. W. Locasale and L. C. Cantley, *Cell Metab.*, **14**, 443 (2011).
29. F. Zagari, M. Jordan, M. Stettler, H. Broly and F. M. Wurm, *N. Biotechnol.*, **30**, 238 (2013).
30. A. Ghorbaniaghdam, J. Chen, O. Henry and M. Jolicoeur, *PLoS One*, **9**, e90832 (2014).
31. N. Ma, J. Ellet, C. Okediadi, P. Hermes, E. McCormick and S. Casnocha, *Biotechnol. Prog.*, **25**, 1353 (2009).
32. J. Wahrheit, J. Niklas and E. Heinzle, *Metab. Eng.*, **23**, 9 (2014).
33. Y. T. Sun, L. Zhao, Z. Ye, L. Fan, X. P. Liu and W. S. Tan, *Biochem. Eng. J.*, **81**, 126 (2013).
34. H. Le, S. Kabbur, L. Pollastrini, Z. Sun, K. Mills, K. Johnson, G. Karypis and W. S. Hu, *J. Biotechnol.*, **162**, 210 (2012).
35. N. Templeton, J. Dean, P. Reddy and J. D. Young, *Biotechnol. Bioeng.*, **110**, 2013 (2013).
36. J. Yong, H. Bischof, S. Burgstaller, M. Siirin, A. Murphy, R. Malli and R. J. Kaufman, *Elife*, **8**, e49682 (2019).
37. M. C. Klein, K. Zimmermann, S. Schorr, M. Landini, P. A. W. Klemens, J. Altensell, M. Jung, E. Krause, D. Nguyen, V. Helms, J. Rettig, C. F. Trost, A. Cavalié, M. Hoth, I. Bogeski, H. E. Neuhaus, R. Zimmermann, S. Lang and I. Haferkamp, *Nat. Commun.*, **9**, 3489 (2018).
38. H. C. Yoo, S. J. Park, M. Nam, J. Kang, K. Kim, J. H. Yeo, J. K. Kim, Y. Heo, H. S. Lee, M. Y. Lee, C. W. Lee, J. S. Kang, Y. H. Kim, J. Lee, J. Choi, G. S. Hwang, S. Bang and J. M. Han, *Cell Metab.*, **31**, 267 e12 (2020).
39. H. C. Yoo, Y. C. Yu, Y. Sung and J. M. Han, *Exp. Mol. Med.*, **52**, 1496 (2020).
40. J. D. Budge, T. J. Knight, J. Povey, J. Roobol, I. R. Brown, G. Singh, A. Dean, S. Turner, C. M. Jaques, R. J. Young, A. J. Racher and C. M. Smales, *Metab. Eng.*, **57**, 203 (2020).
41. W. Li, Z. Fan, Y. Lin and T. Y. Wang, *Front. Bioeng. Biotechnol.*, **9**, 646363 (2021).
42. S. M. Houten, S. Violante, F. V. Ventura and R. J. Wanders, *Annu. Rev. Physiol.*, **78**, 23 (2016).
43. J. M. Gutierrez, A. Feizi, S. Li, T. B. Kallehauge, H. Hefzi, L. M. Grav, D. Ley, D. B. Hizal, M. J. Betenbaugh, B. Voldborg, H. F. Kildegaard, G. M. Lee, B. O. Palsson, J. Nielsen and N. E. Lewis, *Nat. Commun.*, **11**, 68 (2020).
44. K. Hiller, A. Grote, M. Scheer, R. Munch and D. Jahn, *Nucleic Acids Res.*, **32**, 375 (2004).
45. N. Fankhauser and P. Maser, *Bioinformatics*, **21**, 1846 (2005).
46. Z. Yang, A. Halim, Y. Narimatsu, H. J. Joshi, C. Steentoft, K. T. B. G. Schjoldager, M. A. Schulz, N. R. Sealover, K. J. Kayser, E. P. Bennett, S. B. Levery, S. Y. Vakhrushev and H. Clausen, *Mol. Cell Proteomics.*, **13**, 3224 (2014).
47. L. D. Kapp and J. R. Lorsch, *Annu. Rev. Biochem.*, **73**, 657 (2004).
48. S. M. Noh, M. Sathyamurthy and G. M. Lee, *Curr. Opin. Chem.*, **2**, 391 (2013).
49. B. Tihanyi and L. Nyitray, *Drug Discov. Today Technol.*, **38**, 25 (2021).
50. L. D. David Reinhart, W. Sommeregger, A. Gili, S. Schafellner, A. Castan, C. Kaisermayer and R. Kunert, *BMC Proc.*, **9**, 36 (2015).
51. S. H. Kim and G. M. Lee, *Appl. Microbiol. Biotechnol.*, **74**, 152 (2007).
52. M. K. Jeon, D. Y. Yu and G. M. Lee, *Appl. Microbiol. Biotechnol.*, **92**, 779 (2011).
53. S. M. Noh, J. H. Park, M. S. Lim, J. W. Kim and G. M. Lee, *Appl. Microbiol. Biotechnol.*, **101**, 1035 (2017).
54. N. Irani, M. Wirth, J. V. D. Heuvel and R. Wagner, *Biotechnol. Bioeng.*, **66**, 238 (1999).
55. C. Vallee, Y. Durocher and O. Henry, *J. Biotechnol.*, **169**, 63 (2014).
56. C. Toussaint, O. Henry and Y. Durocher, *J. Biotechnol.*, **217**, 122 (2016).
57. S. K. Gupta, S. K. Srivastava, A. Sharma, V. H. H. Nalage, D. Salvi, H. Kushwaha, N. B. Chitnis and P. Shukla, *PLoS One*, **12**, e0181455 (2017).
58. S. K. Gupta, A. Sharma, H. Kushwaha and P. Shukla, *Front. Pharmacol.*, **8**, 463 (2017).
59. K. F. Wlaschin and W. S. Hu, *J. Biotechnol.*, **131**, 168 (2007).
60. D. S. Leong, J. G. Tan, C. L. Chin, S. Y. Mak, Y. S. Ho and S. K. Ng, *Sci. Rep.*, **7**, 45216 (2017).
61. D. S. Z. Leong, B. K. H. Teo, J. G. L. Tan, H. Kamari, Y. S. Yang, P. Zhang and S. K. Ng, *Sci. Rep.*, **8**, 4037 (2018).
62. C. A. Wilkens, C. Altamirano and Z. P. Gerdtzen, *Biotechnol. Bioprocess Eng.*, **16**, 714 (2011).
63. M. Torres, J. Berrios, Y. Rigual, Y. Latorre, M. Vergara, A. J. Dickson and C. Altamirano, *Chem. Eng. Sci.*, **205**, 201 (2019).
64. M. Buchsteiner, L. E. Quek, P. Gray and L. K. Nielsen, *Biotechnol. Bioeng.*, **115**, 2315 (2018).
65. J. Moller, K. Bhat, L. Guhl, R. Portner, U. Jandt and A. P. Zeng, *Eng. Life Sci.*, **21**, 100 (2021).
66. L. T. Urquiza, A. E. A. Martin, D. C. James, T. Nagy and R. J. Falconer, *Biotechnol. Prog.*, **36**, e2940 (2020).
67. L. Scorrano, M. A. De Matteis, S. Emr, F. Giordano, G. Hajnóczky, B. Kornmann, L. L. Lackner, T. P. Levine, L. Pellegrini, K. Reinisch, R. Rizzuto, T. Simmen, H. Stenmark, C. Ungermann and M. Schuldiner, *Nat. Commun.*, **10**, 1287 (2019).
68. L. L. Calleja, M. Lecina, J. L. Repullo, J. Albiol, C. Sola and J. J. Cairo,

Appl. Microbiol. Biotechnol., **99**, 9951 (2015).
69. D. Zalai, K. Koczka, L. Parta, P. Wechselberger, T. Klein and C. Herwig, *Biotechnol. Prog.*, **31**, 1657 (2015).
70. J. B. J. J. Osman, J. Varley, *Biotechnol. Bioeng.*, **79**, 398 (2002).
71. M. Brunner, P. Doppler, T. Klein, C. Herwig and J. Fricke, *Eng. Life Sci.*, **18**, 204 (2018).
72. M. Gagnon, G. Hiller, Y. T. Luan, A. Kittredge, J. DeFelice and D. Drapeau, *Biotechnol. Bioeng.*, **108**, 1328 (2011).
73. B. C. Mulukutla, J. Kale, T. Kalomeris, M. Jacobs and G. W. Hiller, *Biotechnol. Bioeng.*, **114**, 1779 (2017).
74. C. Calmels, A. McCann, L. Malphettes and M. R. Andersen, *Metab Eng.*, **51**, 9 (2019).
75. B. B. Yahia, L. Malphettes and E. Heinzle, *Appl. Microbiol. Biotechnol.*, **99**, 7009 (2015).
76. Z. Huang, D. Y. Lee and S. Yoon, *Biotechnol. Bioeng.*, **114**, 2717 (2017).
77. S. Kyriakopoulos, K. S. Ang, M. Lakshmanan, Z. Huang, S. Yoon, R. Gunawan and D. Y. Lee, *Biotechnol. J.*, **13**, e1700229 (2018).
78. P. Tang, J. Xu, A. Louey, Z. Tan, A. Yongky, S. Liang, Z. J. Li, Y. Weng and S. Liu, *Crit. Rev. Biotechnol.*, **40**, 265 (2020).
79. S. Sha, Z. Huang, Z. Wang and S. Yoon, *Curr. Opin. Chem. Eng.*, **22**, 54 (2018).
80. M. M. Islam, W. L. Schroeder and R. Saha, *Curr. Opin. Syst. Biol.*, **26**, 72 (2021).
81. E. Stalidzans, A. Seiman, K. Peebo, V. Komasilovs and A. Pentjuss, *Biochem. Soc. Trans.*, **46**, 261 (2018).
82. J. M. Gutierrez and N. E. Lewis, *Biotechnol. J.*, **10**, 939 (2015).
83. Z. Rejc, L. Magdevska, T. Trselic, T. Osolin, R. Vodopivec, J. Mraz, E. Pavliha, N. Zimic, T. Cvitanović, D. Rozman, M. Moškon and M. Mraz, *Comput. Biol. Med.*, **88**, 150 (2017).
84. C. Altamirano, A. Illanes, S. Becerra, J. J. Cairo and F. Godia, *J. Biotechnol.*, **125**, 547 (2006).
85. Z. Sheikholeslami, M. Jolicoeur and O. Henry, *Biotechnol. Prog.*, **30**, 535 (2014).
86. C. M. O'Brien, Q. Zhang, P. Daoutidis and W. S. Hu, *Metab Eng.*, **66**, 31 (2021).
87. B. Ben Yahia, L. Malphettes and E. Heinzle, *Metab Eng.*, **66**, 204 (2021).
88. S. Selvarasu, D. Y. Kim, I. A. Karimi and D. Y. Lee, *J. Biotechnol.*, **150**, 94 (2010).
89. Y. Zhanga, H. Lin, Z. Yanga, J. Wanga, Y. Sun, B. Xu and Z. Zhao, *J. Biomed. Inform.*, **99**, 103294 (2019).
90. J. Schmitt, B. Downey, J. Beller, B. Russell, A. Quach, D. Lyon, M. Curran, B. C. Mulukutla and C. Chu, *Biotechnol. Bioeng.*, **116**, 2223 (2019).
91. S. M. Schinn, C. Morrison, W. Wei, L. Zhang and N. E. Lewis, *Biotechnol. Bioeng.*, **118**, 2118 (2021).
92. Z. Huang, J. Xu, A. Yongkyb, C. S. Morrisa, A. L. Polancoa, M. Reilyc, M. C. Borysb, Z. J. Lib and S. Yoon, *Biochem. Eng.*, **160**, 107638 (2020).
93. C. Calmels, S. Arnoult, B. B. Yahia, L. Malphettes and M. R. Andersen, *Metab. Eng.*, **9**, e00097 (2019).
94. Z. Huang and S. Yoon, *Processes*, **8**, 331 (2020).
95. Y. Luo, R. J. Lovelett, J. V. Price, D. Radhakrishnan, K. Barnthouse, P. Hu, E. Schaefer, J. Cunningham, K. H. Lee, R. B. Shivappa and B. A. Ogunnaike, *Biotechnol. J.*, **16**, e2000261 (2021).
96. R. P. van Rosmalen, R. W. Smith, V. A. P. Martins dos Santos, C. Fleck and M. Suarez-Diez, *Metab. Eng.*, **64**, 74 (2021).
97. H. C. Yeo, J. Hong, M. Lakshmanan and D. Y. Lee, *Metab. Eng.*, **60**, 138 (2020).

Jong Youn Baik obtained B.S. and Ph.D. degree in Biological Science from Korea Advanced Institute of Science and Technology (KAIST), Korea in 2003 and 2009, respectively. He was a post-doctoral research fellow at Rensselaer Polytechnic Institute (RPI; 2009-2010), College of Nanoscale Science and Engineering (CNSE; 2010-2013), and University of Delaware (2013-2016) in USA. He was a research associate and research assistant professor in University of Delaware (2016-2019) and an associate site director, UD site in Advanced Mammalian Biomanufacturing Innovation Center (AMBIC; 2016-2019). He joined Inha University in 2019 and is currently an Assistant Professor in Biological Engineering. He has published more than 20 papers and was awarded the grand prize at Korea Biopharmaceuticals Award in 2021.