Cells as a Biofactory: Parallels between Biopharmaceutical Manufacturing and Industrial Biotechnology

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Abstract

Industrial biotechnology and biopharmaceutical manufacturing leverage biology to enable cellular systems to serve as factories to produce molecules of value to humankind. These biotechnological processes utilize diverse host organisms and address applications from biofuel, polymer building blocks, antibiotics, and whole cell therapies. Industrial biotechnology can address environmental and sustainability goals in addition to chemical production. In a similar fashion, the field of biopharmaceutical manufacturing has and continues to produce life-saving medicines. Despite these diverse applications, these fields rely on common biological themes and require similar approaches for genetic and metabolic engineering as discussed in this review. Through advances in synthetic biology, targeted genetic engineering, DNA sequencing, adaptation and high-throughput screening, industrial biotechnology and biopharmaceutical manufacturing utilize the same framework for efficient biochemical production which can be leveraged in current and future collaborations to enable rapid innovation.

1 Introduction

The power of recombinant DNA technology has enabled society to harness biotechnology toward a wide variety of applications, and humanity is still in the early days of realizing these benefits. Even in these early days, different types of cells have demonstrated an ability to solve specific types of problems. For example, microbes, which are diverse in their environments, genes, metabolism, and growth, are particularly useful in addressing applications that benefit from diverse biochemistry and the ability for robust growth. Engineered mammalian cells have very sophisticated abilities to synthesize complex products – from monoclonal antibodies (mAbs) and vaccines to gene therapy vectors. Additionally, mammalian cells can produce complex and human-like post-translational modifications such as glycosylation which are critical for appropriate pharmacological activity. In addition to serving as a biofactory, the cells used in biopharmaceutical applications have gone beyond the factory and are also the medicinal product themselves such as in CAR-T immunotherapies.

Industrial biotechnology, the application of biotechnology to produce commodity and specialty chemicals or recycle waste and plastics, utilizes a broad set of microorganisms as cellular factories. These applications are often (but not always) characterized by high volume, low margin products. Most commonly using bacteria, yeast or fungi, industrial biotechnology leverages central carbon metabolic pathways to enable and enhance production of desired compounds with applications such as biofuels, commodity chemicals and polymer precursors (Curran and Alper, 2012). In addition to chemical production, the use of these organisms extends the possible carbon feedstocks which can be utilized including lignocellulosic biomass and waste oils (Kommoji et al., 2021; Lad et al., 2022). These microbes can help achieve goals in greenhouse gas emission and waste management with potentially concurrent biochemical production (Cordova et al., 2020; Gordillo Sierra et al., 2022; Wongsirichot et al., 2022). Microbes, isolated from nature or engineered

within research labs, can tolerate and sometimes flourish in environments such as high temperature, high pressure, high salt, and the presence of toxic chemicals which are beneficial characteristics for commercial manufacturing (Satyanarayana et al., 2013; Yin et al., 2015; Brabender et al., 2018; Chen and Jiang, 2018; Cordova et al., 2020).

Like industrial biotechnology, biopharmaceutical manufacturing relies on cellular systems toward the goal of producing medicines. Here, however, these processes are often characterized by low volume, high margin products. The resulting compounds can range from small proteins and antibodies to vaccines and even whole cells as the delivery vehicle or therapy. In the case of these biomedicines, extensive clinical studies and regulatory approvals are involved to protect patient outcomes. The complexity of the desired product drives host selection with simpler products produced in bacteria and yeast and more complex proteins, especially those requiring glycosylation, produced in mammalian cell lines. Proper glycosylation is a critical attribute of the drug product and has impacts on drug targeting, *in vivo* activity, and half-life (Ebersbach et al., 2012). Due to the growth in the number of approved monoclonal antibody drugs, Chinese Hamster Ovary (CHO) cells are the most common host with myeloma NS0 and Sp2, BHK-21, and Human Embryonic Kidney (HEK293) cells also leveraged for specific product classes (Lindskog et al., 2018). In addition to monoclonal antibodies, vaccines and cell/gene therapies represent the most established and the newest biopharmaceutical products, respectively (Jagschies, 2018a). Vaccine production uses various host cells depending on the classification and complexity while cell and gene therapies are typically reserved for specialized cell types (Jagschies, 2018b). The use of mammalian cell types, which have slower growth rates as compared to bacteria and yeast, extends the length of experimental design which can be compounded with challenges in application of synthetic biology tools. Nonetheless, the biopharmaceutical manufacturing field has enabled production of diverse medicinal products with at

least four monoclonal antibody products reaching blockbuster status, greater than 1 billion in sales within the first year of release, as of 2016 (Jagschies, 2018b; Spahn et al., 2022).

In both industrial biotechnology and biopharmaceutical manufacturing, post-production processing is a critical step in isolating the desired product from the bioreactor environment of cells and supernatant. These processes can vary widely depending on the characteristics and application of the biochemical produced which requires additional considerations for process scale-up and final economic viability. Unit operations used for downstream processing can include filtration, chromatography, viral inactivation, liquid-liquid extraction, and/or distillation, with several purification steps required to achieve desired purity. Downstream purifications, in either industrial biotechnology or biopharmaceutical manufacturing, represent an entirely separate but related field and are reviewed extensively elsewhere (Cheng et al., 2012; Jungbauer, 2013; Kumar et al., 2019; Mehta, 2019; Datar and Rosén, 2020; Sengupta et al., 2020).

Industrial biotechnology and biopharmaceutical manufacturing represent applications within biotechnology which rely on a biological basis and use the same techniques and approaches (**Figure 1**). Each field has unique characteristics in the production of useful biomolecules as shown in **Table 1**. Specifically, similarities in tools and screening approaches unite industrial biotechnology and biopharmaceutical manufacturing. The synthetic biology toolbox brings genetic engineering closer to an ideal ‘plug and play’ system where parts can be used interchangeably with predictable function across host organisms (Litcofsky et al., 2012). Additionally, genetic engineering techniques including CRISPR and sequencing technologies have drastically improved over the past 20 years enabling more efficient cellular manipulation. With these new techniques for genetic modification, rapid screening has been required to complete the design, build, test, learn cycle to enhance biomolecule production (Petzold et al., 2015; Carbonell et al., 2018). Robotics and automation in addition to assay development have enabled this rapid screening to more rapidly identify top producing strains or

clones (Houston and Banks, 1997; Carbonell et al., 2020; Gurdo et al., 2022). In cases where less information is available, especially in non-conventional hosts, adaptation has been leveraged to enable higher chemical production or tolerance to strenuous environments (Thorwall et al., 2020). Together, these techniques enable successes in both industries and serve as common ground for innovation. Additionally, these industries rely on the same basis of economic feasibility and efficient metabolism which adds to complexity of using biological production systems (Zhang and Hua, 2016; Perin and Jones, 2019; Yang et al., 2020; Nielsen et al., 2022). Balancing the risk of culture contamination with efficient and stable production, biomolecules for chemical or medicinal use require complex understanding and control of biological systems. For successful development of future medicines and biochemicals, continued and additional collaboration efforts can promote innovation toward addressing global challenges of the 21st century related to food, fuel and human health. Industrial biotechnology enables use of non-edible carbon for biofuel processes and can create many commodity chemicals required to replace dependence on non-renewable fossil fuels (Moshelion and Altman, 2015; Dupont-Inglis and Borg, 2018; Yuan et al., 2022). While biopharmaceutical manufacturing has a more targeted range of applications, these still span diverse medical treatments such as life-saving insulin proteins, high-specificity monoclonal antibodies, and even serve as a cure for cancer and genetic diseases (Assidi et al., 2022). This review provides a concise summary of several areas related to cell line/strain development and engineering across both industrial biotechnology and biopharmaceutical manufacturing providing motivation for continued and additional collaboration and innovation between these fields.

2 Parallels between Biopharmaceutical Manufacturing and Industrial Biotechnology

Industrial biotechnology and biopharmaceutical manufacturing rely heavily on cellular systems to serve as ‘biofactories’ which create useful compounds with diverse applications. Regardless of the organism and molecule chosen, production at industrially relevant scales relies on harnessing

biological control at the DNA or RNA level to make genetic modifications. These systems also rely on the same metabolic pathways which govern the movement of carbon from feedstocks to the desired product.

2.1 Advances in Synthetic Biology

Development of synthetic biology tools have enabled a variety of organisms to be used for bio-production. From the building blocks of gene expression (toolbox of parts) to gene editing and DNA sequencing, advances in synthetic biology have enabled enhanced product development for both biopharmaceutical manufacturing and industrial biotechnology applications.

2.1.1 Synthetic Biology Toolkit

At the core of recombinant chemical and biologic production is the genetic engineering of organisms to enable or enhance production. This engineering relies on a toolbox of synthetic biology parts (promoters, terminators, enhancers, etc.) as building blocks to enable efficient gene and protein expression, and ultimately proper protein folding and localization. It is also desirable for these toolbox components to function independently to enable mix-and-match of parts directly 'off the shelf'. As these building blocks serve a critical role across biotechnology applications, significant research has been performed to identify native sequences as well as to create synthetic versions by combining smaller functional units together (Curran et al., 2013; Redden and Alper, 2015; Cheng and Alper, 2016; Cheng et al., 2019). In the case of promoters, transcription factor binding sites and efficient ribosome binding sites have served as the basis for promoter design across all evolutionary scales from *Escherichia. coli* (Chen et al., 2018) and yeast (Redden and Alper, 2015) to CHO cells (Johari et al., 2019) and human cell lines (Cheng and Alper, 2016). As a second most important core element following the target gene, terminators enable fine-tuning of expression often through sequence modification to achieve optimal expression.

143 Promoters are perhaps the most well studied component of the synthetic biology toolbox.
144 Engineering of these systems leads to dramatic changes in gene expression. The ability for induction
145 and tuning of gene expression has been critical in finding a balance between conflicting needs of
146 growth and protein or chemical production. As such, discovery and development of promoters is
147 required in both industrial biotechnology and biopharmaceutical manufacturing applications across a
148 variety of host organisms (**Table 2**). The length and structural complexity of promoters increases
149 with evolutionary complexity with several key features outlined for bacteria, yeast, and mammalian
150 production hosts (**Table 2**). Mining of native promoter sequences has been applied to microbial and
151 mammalian cultures to leverage innate transcription factor binding motifs (Cheng and Alper, 2016;
152 Johari et al., 2019; McGraw et al., 2020). The conserved presence of these factors enables a pipeline
153 approach which can be applied to any organism of interest for any application (Redden and Alper,
154 2015; Cheng and Alper, 2016; Johari et al., 2019). Specifically, in mammalian cells the transcription
155 factor, Nuclear Factor κB (NF- kappaB) is present within the CMV promoter routinely used in
156 biopharmaceutical applications (McGraw et al., 2020). While this factor is absent in yeast, a similar
157 function is completed through retrograde response genes (Srinivasan et al., 2010). In addition to
158 conserved binding motifs, features including inducibility and multi-expression cassettes are required
159 and desired in all production hosts for biochemical production. In many cases, a core promoter
160 sequence can be multiplexed with upstream activating sequences from other promoter sequences to
161 generate novel sequences. Continued study and development of promoter sequences, especially for
162 mammalian cells, is essential for expanding the synthetic biology toolbox to achieve tighter
163 expression control with shorter sequences to ultimately improve production.

164 Similar approaches building up from various motifs have been applied to terminator
165 development. Although these regions are less studied, they are critical in fine-tuning mRNA half-life
166 to achieve optimal gene expression and have been engineered across all production hosts (Morse et

al., 2017; Cheng et al., 2019). In deploying the same sequence structure, a range of terminators have been developed within *Saccharomyces cerevisiae* and human cell line lines in parallel demonstrating a wide range of activity (Morse et al., 2017; Cheng et al., 2019). These examples highlight the DNA molecular basis of synthetic biology parts which is conserved across applications in industrial biotechnology and biopharmaceutical manufacturing. While promoters and terminators are considered most critical in controlling gene expression, they represent only two parts of the synthetic biology toolbox. For brevity, repressors, insulators, activators and enhancers are not discussed here and are reviewed extensively elsewhere (Gaszner and Felsenfeld, 2006; Moore et al., 2014; Shlyueva et al., 2014; Engstrom and Pfleger, 2017; Hafner and Boettiger, 2022). With continued development of diverse synthetic biology tools, open-source projects have sought to provide standardization in storage of data, organization of tools, and reference software for routine analysis (Decoene et al., 2017). These efforts including the Synthetic Biology Open Language (SBOL) (Decoene et al., 2017), SynBioHub (McLaughlin et al., 2018), and SynBiopython (Yeoh et al., 2021) provide tools and information across laboratories to speed progress as accessibility enables researchers to build upon the work of others. Continued sharing of synthetic biology toolbox knowledge will be beneficial for both industrial biotechnology and biopharmaceutical manufacturing applications.

2.1.2 Gene Editing including CRISPR

Genetic engineering for industrial biotechnology or biopharmaceutical manufacturing applications relies on efficient delivery of the desired genetic cargo often into a known location within the genome. Discovery of site-specific tools including Zinc Finger Nucleases (ZFN), transcription activator like effector nucleases (TALEN) and CRISPR have enabled this level of precise genetic engineering (Urnov et al., 2010; Copeland et al., 2014; Doudna and Charpentier, 2014; Wright et al., 2014). By leveraging DNA interactions for these approaches, they are broadly applicable across all domains of life with applications in both industrial biotechnology and biopharmaceutical

manufacturing. Specific applications of CRISPR span a large body of literature and genetic editing of *E. coli*, *S. cerevisiae*, and CHO cells are extensively reviewed elsewhere (Peters et al., 2015; Stovicek et al., 2017; Deaner and Alper, 2019; McGraw et al., 2020; Cai et al., 2022).

In addition to direct gene editing, the CRISPR/Cas system has been modified and repurposed in a myriad of ways to serve as a 'molecular Swiss army knife' (Mans et al., 2015). Partial or full deactivation of Cas function has been applied to fine tune behavior for enhanced gene editing (Ran et al., 2013), for tighter control of gene expression or repression (Deaner et al., 2017) and for various RNAi strategies (Schultenkämper et al., 2019) across all cellular workhorses and product applications. Additionally, CRISPR based approaches generating DNA nicks have been used for enhanced site-specific integration and mutation generation for screening purposes (Halperin et al., 2018; Hamaker and Lee, 2023). Gene editing tools are central to all biotechnology applications and serve as a common thread between the industrial biotechnology and biopharmaceutical manufacturing fields.

2.1.3 DNA Sequencing

Closely tied to genetic editing innovation, advancement of DNA sequencing technology over the past 20 years has propelled biochemical production across all scales of life. Conventional production host genomes such as *E. coli* (4.6 million base pairs) and *S. cerevisiae* (12 million base pairs) were first sequenced and published in 1996 and 1997, respectively, while the Human Genome Project released the first human genome in 2000 (Saccharomyces Genome Database | SGD, n.d.; Blattner et al., 1997; Engel et al., 2014; Nurk et al., 2022). The complete sequence and assembly required another 20 years to complete in part due to additional technological advances enabling longer, high-fidelity sequencing reads (Nurk et al., 2022). Development of sequencing technologies using parallel reactions have enabled high fidelity and longer read lengths with lower costs. Traditional Sanger sequencing is useful for projects where short reads are sufficient, typically less than 1kb (McCombie

et al., 2019). By deploying a sequencing by synthesis approach, detection of the newly added nucleotide can be quantified using direct fluorescence or post-synthesis detection via released phosphate groups or pH changes as used in Illumina and Ion Torrent technology (McCombie et al., 2019). For read lengths exceeding 500 base pairs, single molecule sequencing techniques use nanoscale surfaces for DNA synthesis in PacBio instruments or direct detection in the case of Oxford Nanopore sequencing (McCombie et al., 2019). These technological advances and various methods from Sanger sequencing to Next-Generation Sequencing approaches merit their own independent review which can be found elsewhere in the literature (Grada and Weinbrecht, 2013; Slatko et al., 2018; McCombie et al., 2019; Hu et al., 2021). Specifically, within applications utilizing adaptation, such as adaptive laboratory evolution, DNA sequencing provides key insights and can provided causative mutations for the desired phenotype which can apply to both industrial biotechnology and biopharmaceutical manufacturing (Phaneuf et al., 2019; Sandberg et al., 2019). Likewise, in the case of non-conventional organisms, microbial communities or even those which cannot be cultured *in vivo*, sequencing enables genome mining providing critical insight and potentially novel enzymes (Seppälä et al., 2017; Panaiotov et al., 2021). Specifically, within biopharmaceuticals, full genome assembly has been challenging for the main workhorse, CHO cells. Within the past 5 years, small molecule real time sequencing paired with extensive scaffolding has generated the most up-to-date Chinese Hamster genome assembly with 97% sequence coverage for direct use in CHO cell applications (Rupp et al., 2018; Hilliard et al., 2020). Continued development of DNA sequencing technology will speed data collection, reduce analysis times, and enable more robust annotation and assembly to enable rapid studies of cellular systems for biochemical production.

2.2 Strain/Clone Development

A critical aspect of the metabolic engineering design, build, test cycle is evaluation of strains or clones to identify the best producer. This process can be called strain development for microbial

hosts or clone development for mammalian hosts and leverages adaptation and high-throughput screening. These tools find the 'needle in a haystack' cell which may have the appropriate genetic modification, desired enzyme mutation, or even higher tolerance to process impurities. From traditional approaches utilizing random integration of transgenes to more targeted point mutations, screening is required to isolate the desired phenotype and corresponding cell(s). In both industrial biotechnology and biopharmaceutical manufacturing, single cell/strain cloning is critical for consistent growth and biochemical reproducibility. Specifically, within biopharmaceutical manufacturing, clone screening is needed to isolate a homogenous population of favorable product quality attributes such as glycosylation or charge variants. In addition to finding desired rare events, strain/clone development is required to ensure an optimal production host. While traditional strain/cell line development is performed *in vivo*, recent work is leveraging cell-free or hybrid approaches for biomolecule production (Luro et al., 2019; Silverman et al., 2019). These systems enable construction of complex pathway networks using cell lysates which can be rapidly multiplexed via mixing as compared to extensive cellular engineering (Luro et al., 2019; Silverman et al., 2019). Whether performed *in vivo* or *in vitro*, development of cell lines/strains is critical to creating platforms for biomolecule production.

2.2.1 Adaptation

Adaptation is a widely used technique which leverages natural or induced mutations to create diverse cellular populations and achieve desired behaviors in industrial biotechnology and biopharmaceutical manufacturing. Often employed when direct mechanisms are not known, adaptation enables cells to be selected based on an externally desirable phenotype. Regularly employed in industrial biotechnology applications, adaptation or adaptive laboratory evolution (ALE) utilizes growth-based selection to improve growth or tolerance to inhibitory compounds. Adaptation is typically performed on shorter time frames with longer studies (30+ growth cycles) designated as ALE. Inhibitory

compounds can range from metabolic overflow products to by-products of lignocellulosic biomass and waste carbon sources for recycling (Walker et al., 2019; Wang et al., 2020; Coleman et al., 2023; Mavrommati et al., 2023). Implementation of ALE has increased ethanol tolerance for higher production yields within *S. cerevisiae* and improved methanol tolerance/conversion in the methanol-dependent methylotroph *Corynebacterium glutamicum* (Walker et al., 2019; Wang et al., 2020). In a similar approach, tolerance to toxic compounds such as phenol and furfural has been achieved in a variety of organism hosts including *Escherichia coli*, *Kluyveromyces marxianus*, *Yarrowia lipolytica*, and *Zymomonas mobilis* (Shui et al., 2015; Hemansi et al., 2022; Zheng et al., 2022; Coleman et al., 2023). In situations where additional mechanisms are known, directed evolution can be applied where targeted mutations are introduced rather than relying on genome wide, random modifications. Directed evolution is commonly deployed for enzymes or other small molecule screening to improve or diversify function (Romero and Arnold, 2009; Arnold, 2018). Regardless of the approach, industrial biotechnology has and continues to leverage the benefits of evolution-based approaches toward achieving production goals.

In a parallel manner, the biopharmaceutical manufacturing industry has a long history of applying adaptation to identify cell lines with fast growth in suspension culture and the appropriate selection markers (Dahodwala and Lee, 2019). Specifically, within CHO cells, adaptation schemes with random and chemical mutagenesis have enabled methotrexate and glutamine synthase selection systems which enrich for cell lines with higher expression of the target protein (Dahodwala and Lee, 2019; Szkodny and Lee, 2022). In these cases, adaptation created cell lines which can be further manipulated using selection markers and the synthetic biology tools discussed above. For high level production, various cell line types including but not limited to CHO, HEK293, and Vero cells, have been adapted from adherent to suspension growth conditions to support high cell densities (Shridhar et al., 2017; Rourou et al., 2019; Lomba et al., 2021). Adaptation has been deployed to achieve a

wide variety of cellular phenotypes in biopharmaceutical manufacturing and industrial biotechnology.

2.2.2 High-Throughput Screening

Following genetic editing and/or adaptation, screening is required to identify the best production clone or strain. With advances in genetic editing and tools, the population size for screening efforts has dramatically increased while a shorter time from discovery to market is desired requiring the deployment of high-throughput approaches. High-throughput screening includes miniaturization of cell cultures with rapid analytics to measure all appropriate parameters (Leavell et al., 2020). Across biopharmaceutical manufacturing and industrial biotechnology, high product titer, yield and productivity are critical parameters for optimization. Product quality attributes such as post-translational modifications are also important parameters for biopharmaceutical screening while industrial biotechnology applications may focus on robust growth using non-traditional substrates as another screening metric. Considering the miniaturization of cell culture, new technologies can monitor cells from µL to 250 mL scale conditions utilizing microfluidic devices and unique geometry to create accurate scale-down models (Long et al., 2014; Totaro et al., 2021). In a similar fashion, parallelization and development of process analytical technology has enabled screening of larger populations providing additional depth of data critical for making informed decisions (Long et al., 2014; Leavell et al., 2020). For rapid analytics, microtiter plates were historically used with fluorescence or spectroscopic measurements (Lai et al., 2013; Long et al., 2014; Zeng et al., 2020). Newer data analytics have enabled biosensor detection, electrochemical based sensors and mass spectrometry approaches depending on the size and characteristics of the analyte. Robotics and advanced automation including liquid handler systems have revolutionized the capacity and speed of high-throughput screening (Zeng et al., 2020). The use of simple, modular components enables these devices to be readily deployed in industrial biotechnology applications to measure online parameters

such as temperature, cell density and fluorescence (Wong et al., 2018; Steel et al., 2020). Operating at a 12-25 mL scale, the Chi. Bio system paired with eVOLVER technology can monitor and control multiple miniature reactor vessels from a single computer (Wong et al., 2018; Steel et al., 2020). While this technology has been deployed for continuous evolution applications, it can also be utilized for high-throughput analysis of other parameters critical in strain development (Wong et al., 2018; Steel et al., 2020). In addition, deployment of microfluidics has enabled a variety of screening approaches including live-cell fluorescence imaging (Luro et al., 2019; Bowman et al., 2021). Advanced microfluidic devices can monitor growth of individual cells with isolation of desired clones for both industrial biotechnology and biopharmaceutical applications. These devices use carefully controlled fluid movement to screen and select *E. coli* in the case of the SIFT system and mammalian cell lines in the Berkeley Lights Beacon system (Delivering digital cell biology at light speed, n.d.; Luro et al., 2019). These developments in high-throughput screening have improved biotechnological production in both industrial and biopharmaceutical manufacturing applications using a variety of production hosts. Bringing detection of key product attributes to real or near-real time enables more rapid and informed decision making about the screening process, from how many clones to keep to identifying the optimal conditions. Adaptation and high-throughput screening serve as critical upstream development techniques which are ubiquitously employed across the industrial biotechnology and biopharmaceutical manufacturing industries to identify the most effective cell factories.

3 Discussion

Leveraging a strong biological basis, industrial biotechnology and biopharmaceutical manufacturing rely on similar concepts, methods and techniques. Additionally, advances in synthetic biology, sequencing technology and high-throughput screening have enabled rapid progress in the past 20

years resulting in a variety of new products. The biochemicals or even cells themselves which come out of genetic engineering projects have diverse applications related to food, fuel, and human health.

3.1 Mechanisms for Cross-Disciplinary Innovation

For continued advancement of biotechnology, interdisciplinary collaborations between academics and industry partners are becoming more common. These projects or groups expand the available expertise and enable participants to leverage skills and equipment they might not otherwise be able to access. Within the industrial biotechnology realm, several consortia groups have been created in the past five years to promote and further develop technology. These include the Agile BioFoundry which is a collection of national laboratories focused on bioenergy technologies and BioMADE which is focused on US-based bioindustrial manufacturing (About BioMADE — BioMADE, n.d.; About the Agile BioFoundry, n.d.). The Agile BioFoundry hosts centralized equipment for automated strain building and screening at national laboratories to support academic and industry research projects. These efforts are designed to forward research progress but can also be leveraged as technology and expertise hubs during pandemic crises (Vickers and Freemont, 2022). Unification of several biofoundries into the Global Biofoundry Alliance network connects the Agile BioFoundry and other US-based laboratories with worldwide facilities in Europe, Asia, and Australia (Hillson et al., 2019; Vickers and Freemont, 2022). Likewise, within the biopharmaceutical manufacturing space, two consortia groups known as the Advanced Mammalian Biomanufacturing Innovation Center (AMBIC) and the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) were formed within the past eight years (About NIIMBL, n.d.; What is AMBIC? - AMBIC, n.d.). Despite variation in size and key stakeholders, these consortia groups host dedicated platforms for innovation and connect research groups across multiple biotechnology subfields. More specifically, toward the goal of improving biomanufacturing and the transition of technologies from ideation to practice, BioMADE and NIIMBL have each released detailed information about

Technology Readiness Levels (Kedia et al., 2022; Smanski et al., 2022). These documents create a standard across the industrial biotechnology and biopharmaceutical manufacturing fields which enables clear communication and highlights the steps required to bring new technologies to market (Kedia et al., 2022; Smanski et al., 2022). Additionally, these two sister consortia groups are focused on increasing American manufacturing of biochemicals and biopharmaceuticals while expanding the specialized workforce needed in biotechnological applications. Efforts such as the Agile BioFoundry, AMBIC, BioMADE, and NIIMBL provide a connection between academic and industry researchers to drive faster innovation in biotechnology.

The examples provided here represent just a fraction of the activity by organizations and consortia around the globe. While these groups represent tremendous progress in connection of academic and industrial researchers, few connect progress in industrial biotechnology and biopharmaceutical manufacturing together despite the commonalities outlined here. Sharing of technologies and best practices for cell line/strain screening across projects would enable more efficient processes for both biopharmaceutical manufacturing and industrial biotechnology applications. The general process development steps are maintained and can be automated in nearly identical ways. Likewise, discovery and construction of synthetic biology tools can be shared for more efficient protein expression, whether as the direct product or key catalyst in biochemical production. With additional collaboration through these groups and new partnerships, future innovation can bridge industrial biotechnology and biopharmaceutical manufacturing to more rapidly engineer cells as efficient biofactories.

3.2 Figures

Figure 1: *Contributions from advances in the synthetic biology toolbox and strain/clone development enable cellular systems to be used as a biological-based factory spanning products within industrial biotechnology and biopharmaceutical manufacturing.* Additional collaboration and innovation between these two fields can leverage similarities to achieve ambitious goals in biochemical production.

3.3 Tables

Table 1: *Characteristics of industrial biotechnology and biopharmaceutical manufacturing in producing useful biomolecules*

Industrial Biotechnology	**Biopharmaceuticals**
Extensive synthetic biology tools available	Viral based synthetic biology tools available
Achieved using bacterial and yeast hosts	Achieved using bacterial, yeast and mammalian hosts
Well established genome sequences for conventional hosts	Human and CHO genome sequences available within the past 20 years
Product is small molecules and proteins (lower sale price)	Product is large proteins and/or cells (higher sale price)
Ability to use and/or detoxify waste carbon sources	Established production from chemically-defined growth media
Faster growth rates, shorter process length, Lower risk of contamination	Generate complex human-like glycosylation patterns
Can address global challenges in food, fuel, and commodity chemicals	Can address global challenges in medicine
Selected Examples: 1,3-propanediol, citric acid, biofuels, succinic acid, itaconic acid	Selected Examples: Recombinant insulin, monoclonal antibodies, vaccines, cell & gene therapies

390 Table 2: *Characteristics of selected promoters available within the synthetic biology toolbox for genetic engineering across production hosts*

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Promoter Characteristics	**Bacterial**			**Fungal**			**Mammalian**		
	Promoter engineering for native or synthetic promoters leverages transcription factor binding motifs								
	Shorter in sequence			Sequence length can vary			Longer in sequence		
	Poly-cistronic operons			Multi-expression cassette possible using 2A linker sites			Dual expression possible: IRES for small proteins, large proteins require independent cassettes		
	Tunable & inducible expression readily achieved			Tunable & inducible expression readily achieved in *S. cerevisisae*, more challenging in non-conventional hosts			Limited options for tuning & induction capability		
Selected examples:	T7 promoter	Lac	Trp	SV40	GPD	Ef1α (TEF)	Cytomegalovirus (CMV-IE variant)	SV40	Ef1α (TEF)
Host organisms	Bacteriophage	*Escherichia coli*	*Escherichia coli*	Simian virus expressed in *Saccharomyces pombe*	*Saccharomyces cerevisiae*	*Yarrowia lipolytica*	Cytomegalovirus	Simian Virus	*Cricetulus griseus* (CHO)
Sequence length	18	61	86	420	655	404	2105	420	1335
Viral or Native Origin	Viral	Native	Native	Viral	Native	Native	Viral	Viral	Native
Reference	(T7 Promoter System, n.d.)	(Czarniecki et al., 1997)	(Bass and Yansura, 2000)	(Jones et al., 1988)	(Redden and Alper, 2015)	(Blazeck et al., 2011)	(Cheng and Alper, 2016)	(Das et al., 1985)	(Wang et al., 2017)

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4 Additional Requirements

Number of Figures: 1

Number of Tables: 2

Contribution to the field statement:

Industrial biotechnology and biopharmaceutical manufacturing leverage biology to enable cellular systems to serve as factories to produce molecules of value to humankind. These biotechnological processes utilize diverse host organisms and address applications from biofuel, polymer building blocks, antibiotics, monoclonal antibodies, and whole cell therapies. In this review, areas of foundational research which are used in both industrial biotechnology and biopharmaceutical manufacturing are discussed. Development of synthetic biology tools, DNA editing, and next generation sequencing has enabled genetic engineering across a variety of production organisms. Likewise, strain and clone development aids in identification of the optimal cells for a given application and utilizes approaches in adaptation, high-throughput screening and automation. Together these methods, tools, and techniques enable cellular systems to be used as biological based factories. While many consortia groups have formed in the past 10 years to support innovation with specific focus areas, more collaboration is needed at the intersection of biopharmaceutical manufacturing and industrial biotechnology to share technological advances and best practices.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

LC and KL conceptualized the idea. LC wrote the manuscript. All authors contributed to manuscript revision and approved the submitted version.

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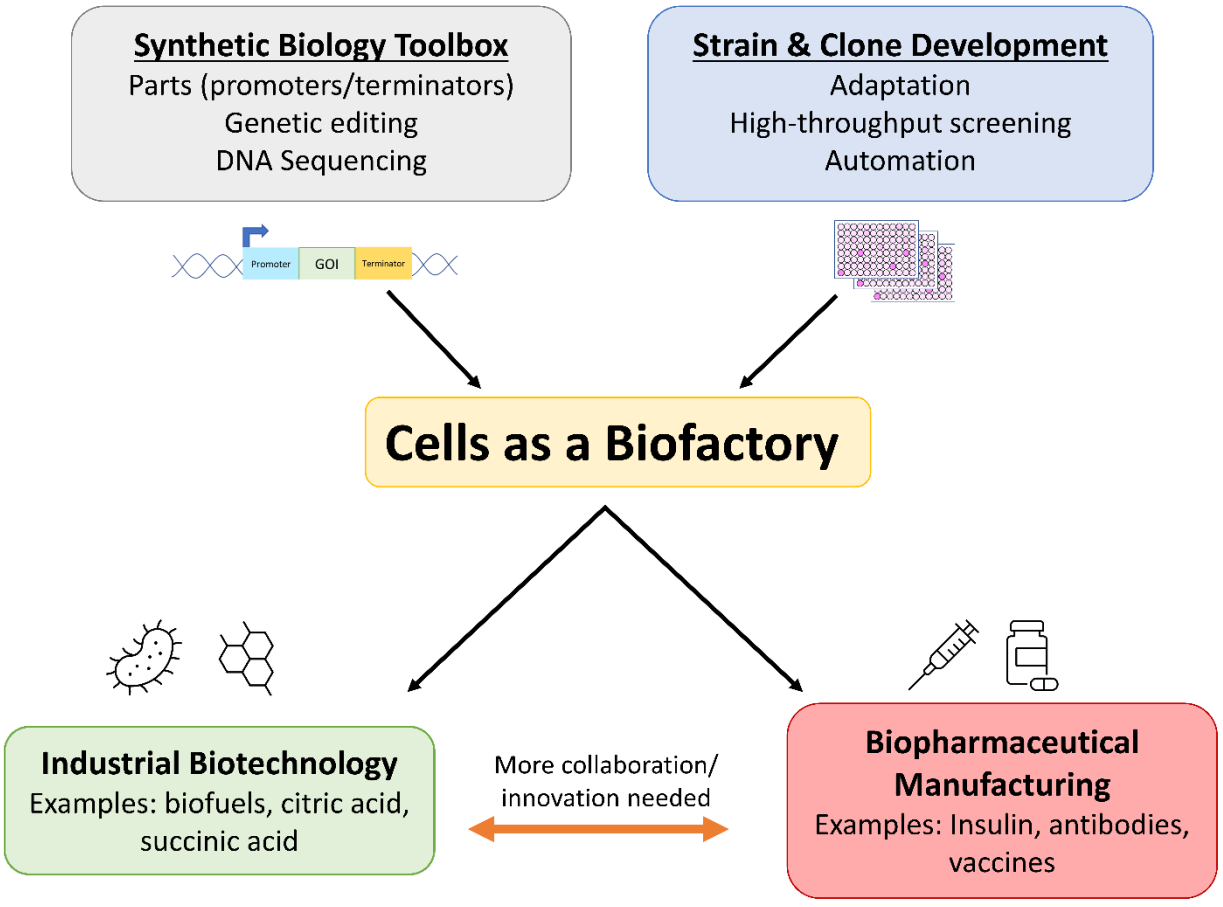
Synthetic Biology Toolbox
Parts (promoters/terminators)
Genetic editing
DNA Sequencing
Strain & Clone Development
Adaptation
High-throughput screening
Automation
Promoter
GOI
Terminator
Cells as a Biofactory
Industrial Biotechnology
Examples: biofuels, citric acid, succinic acid
More collaboration/ innovation needed
Biopharmaceutical Manufacturing
Examples: Insulin, antibodies, vaccines