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Metabolic systems biology and multi-omics of cyanobacteria: Perspectives and future directions

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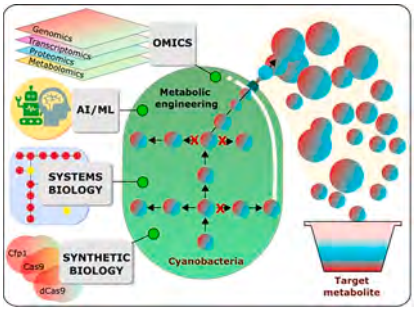
HIGHLIGHTS

- Various techniques for understanding cyanobacterial metabolism are reviewed here.
- Integration of multi-omics data with metabolic models are discussed.
- Various applications of metabolic systems biology and multi-omics are presented.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyanobacteria are oxygenic photoautotrophs whose metabolism contains key biochemical pathways to fix atmospheric CO_2 and synthesize various metabolites. The development of bioengineering tools has enabled the manipulation of cyanobacterial chassis to produce various valuable bioproducts photosynthetically. However, effective utilization of cyanobacteria as photosynthetic cell factories needs a detailed understanding of their metabolism and its interaction with other cellular processes. Implementing systems and synthetic biology tools has generated a wealth of information on various metabolic pathways. However, to design effective engineering strategies for further improvement in growth, photosynthetic efficiency, and enhanced production of target biochemicals, in-depth knowledge of their carbon/nitrogen metabolism, pathway fluxe distribution, genetic regulation and integrative analyses are necessary. In this review, we discuss the recent advances in the development of genome-scale metabolic models (GSMMs), omics analyses (metabolomics, transcriptomics, proteomics, fluxomics), and integrative modeling approaches to showcase the current understanding of cyanobacterial metabolism.

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1. Introduction

The rapidly growing human population and expanding industrialization have increased energy demand and expedited the consumption of natural resources. This overexploitation of natural resources has led to severe damage to the environment and threatened an impending global energy crisis soon. Therefore, there is an increasing demand to design sustainable and eco-friendly strategies to produce sustainable fuel and chemical alternatives (Luan and Lu, 2018). In this context, several native microbes and their engineered variants have been effectively used to produce various renewable biofuels and biochemicals. However, most of these systems are based on heterotrophic microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae*, which require organic carbon sources (glucose or sugar-based feedstocks) for their growth (Dodds, 2002; Jojima et al., 2010; Wang et al., 2019). On the other hand, cyanobacteria can utilize atmospheric carbon to produce biomass through photosynthesis (Singh et al., 2016). Furthermore, marine cyanobacteria offer additional sustainability advantages as they do not need fresh water-based media to grow (Merlo et al., 2021). The recently identified fast-growing strains such as *Synechococcus elongatus* UTEX 2973 (Yu et al., 2015), *Synechococcus elongatus* PCC 11801 (Jaiswal et al., 2018b), *Synechococcus elongatus* PCC 11802 (Jaiswal et al., 2020), *Synechococcus elongatus* PCC 11901 (Włodarczyk et al., 2020), and *Synechococcus elongatus* BDU 130192 (Pathania and Srivastava, 2021) have further enriched the list of potential cyanobacterial strains for biotechnological purposes. In addition to growth advantages, these strains also exhibit valuable properties such as higher biomass accumulation and higher tolerance towards various abiotic factors.

Cyanobacteria contain the biochemical pathways necessary to convert solar energy into biochemical energy to produce a wide variety of energy-rich compounds. Considering the benefits offered by cyanobacteria, many researchers have established and employed metabolic engineering tools to modify native pathways and express heterologous pathways to divert the photosynthetic carbon flux towards value-added bioproducts such as alcohols, hydrocarbons, isoprenoids, sugars, etc. (Knoot et al., 2018; Pattharaprachayakul et al., 2020).

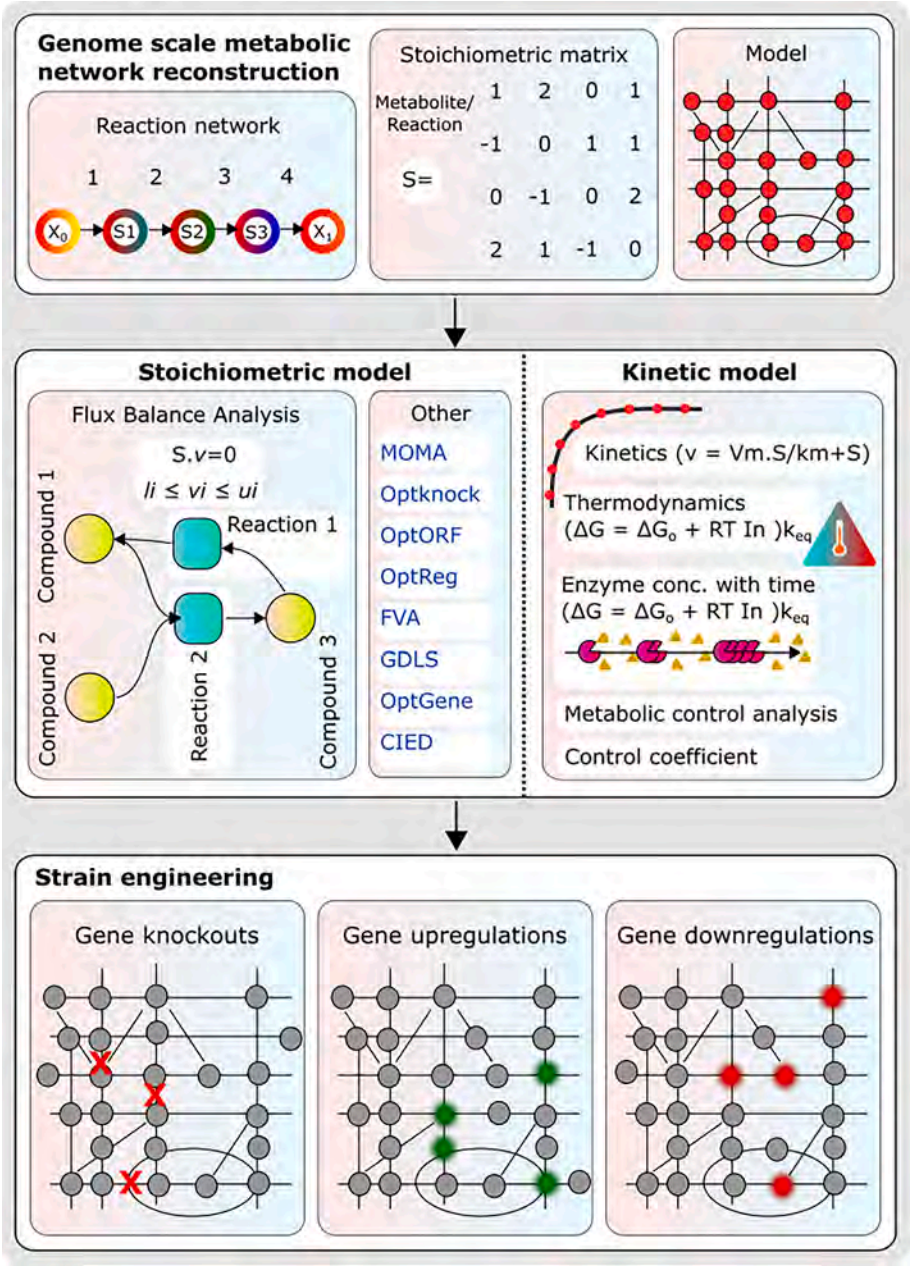

Fig. 1. Strain designing through the application of metabolic models. The diagram illustrates different steps involved in metabolic systems biology that utilizes an integration of computational automation, systems biology, and synthetic biology tools to guide engineering strategies for creating superior cyanobacterial strains. In the lowermost panel, the upregulated gene products (metabolites) are indicated by green color whereas the downregulated gene products are indicated by red (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

1.1. Bottlenecks in research and recent approaches to resolve them

It has been realized that improving the production of native and/or non-native molecules of interest will require extensive manipulation of their genetic and metabolic makeup. Such issues prompt a detailed understanding of cyanobacterial metabolism and its regulation at different levels as well as to generate a comprehensive picture of the regulation. Additionally, such information is required to efficiently develop the newly-identified promising strains as cyanobacterial biofactories. Thus, it is essential to systematically find and remove the metabolic and regulatory blockages, waste cycles or pathways that are responsible for reducing the productivity of desired compounds (Hagemann and Hess, 2018).

These requirements necessitated the development of novel and integrative strategies. Among the recent developments in metabolic engineering of cyanobacteria, the incorporation of the "Design-Build-Test-Learn" (DBTL) paradigm has been explicitly helpful in the optimization of metabolic output and flux distribution (Carbonell et al., 2018; HamediRad et al., 2019). The DBTL pipeline implements a robust integration of computational automation, systems and synthetic biology tools to manipulate the metabolic network for designing cyanobacterial strains (Fig. 1). Various synthetic biology innovations and multi-omics studies such as randomly barcoded transposon insertion have been adapted to identify essential genes and assign gene functions while CRISPR-interference (CRISPRi) and Antisense Expression technologies have been implemented to regulate the expression of target genes to design cyanobacterial strains as hosts for biotechnological applications (Table 1).

The rapidly increasing number of genome sequences and emerging gene-editing technologies such as CRISPR/Cpf1 are opening up new possibilities to explore and engineer cyanobacterial metabolism. Their systematic integration with metabolic systems biology will further empower the engineering programs of cyanobacterial strains. This review provides an overview of emerging applications and significance of metabolic systems biology approaches to delineate the metabolic capabilities of cyanobacteria and their flux control.

2. Genome annotation, reconstruction of genome-scale metabolic models (GSMMs), and their applications in cyanobacterial strain designing

High-throughput (formerly 'next-generation') sequencing techniques (NGS) (Koboldt et al., 2013) such as Illumina dye sequencing, pyrosequencing, SMRT (Single-molecule real-time) sequencing, and nanopore technologies have been developed to sequence and analyse genomes. The rapid and cost-effective genome sequencing techniques have enabled the identification and characterization of several cyanobacterial genomes each year. Various genome annotation pipelines such as the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016), Prokka (prokaryotic annotation) (Seemann, 2014), RAST (Rapid Annotations using Subsystem Technology) (Overbeek et al., 2014), DRAM (Distilled and Refined Annotation of Metabolism) (Shaffer et al., 2020), etc. are available for genome annotation of a wide variety of organisms. Various databases for cyanobacteria have been developed, having information about complete genome sequences, gene annotations, gene information, and species information (e.g., CyanoBase) (Fujisawa et al., 2017), CYORF (Furumichi et al., 2002) etc.

The availability of genome sequences and accurate annotation information represents the first step towards reconstructing genome-based metabolic models that help to understand the primary metabolism of cyanobacteria and its regulation through systems-level analysis (Broddrick et al., 2016). GSMMs are stoichiometry-based structural models which provide information about metabolic networks and don't require information on enzyme kinetic parameters (Gu et al., 2019). These models can predict growth rates, product formation, theoretical yield, and intracellular flux distributions, depending on the constraints and steady-state assumptions. The GSMMs are often combined with additional experimental measurements, such as growth rate, CO_2 and photon uptake rates, O_2 production, etc., to identify the cellular metabolic flux distributions (Vu et al., 2012).

Several automated tools have been developed to reduce the time for reconstruction, such as FAME (Flux Analysis and Modeling Environment) (Boele et al., 2012), Merlin (Metabolic Models Reconstruction using Genome-Scale Information) (Dias et al., 2015), RAVEN (Reconstruction, Analysis, and Visualization of Metabolic Networks) (Agren et al., 2013), ModelSEED (Henry et al., 2010), etc. Methods such as OptORF (Kim and Reed, 2010), OptKnock (Burgard et al., 2003), OptForce (Ranganathan et al., 2010), RobustKnock (Tepper and Shlomi, 2010), etc. have been developed to identify the gene modifications (deletions or overexpression) to form the desired product(s) by analyzing the GSMMs. Tools such as MOMA (minimization of metabolic adjustment) (Segre et al., 2002), RELATCH (minimization of relative metabolic change) (Kim and Reed, 2012) and ROOM (regulatory on/off minimization) (Shlomi et al., 2005) can predict the flux distributions in knockout strains.

In recent years, metabolic modeling has been applied to several cyanobacterial model strains such as *Synechocystis* sp. PCC 6803 (Firoozabadi et al., 2021), *Synechococcus* sp. PCC 7942 (Broddrick et al., 2016), *Synechococcus* sp. PCC 7002 (Hendry et al., 2016), *Cyanothece* sp. ATCC 51142 (Alagesan et al., 2013), and some of the non-model strains, e.g., *Synechococcus elongatus* BDU 130192 (Ahmad et al., 2020). The first metabolic model of *Synechocystis* sp. PCC 6803 was developed by Fu, 2009 and has gone through several upgradations (Sarkar et al., 2019).

The kinetic or dynamic models use thermodynamics, enzyme kinetics, and other metabolic regulatory information to calculate the fluxes or metabolite concentrations (Hameri et al., 2019; Kim et al., 2018). Kinetic modeling with the Metabolic Control Analysis (MCA) approach (Fell, 1992) has been applied to overproduce limonene and ethanol in cyanobacteria (Table 2). Both kinetic modeling and GSMMs have advantages and disadvantages (Table 3).

In recent years, the use of hybrid models has become attractive due to the advantages offered by different types of models to define, calculate and optimize the performance of a biological system. The hybrid models are developed by integrating stoichiometric information and high quality kinetic data and they are evaluated for theoretical and experimental accuracy (Kim et al., 2018). For the creation of hybrid metabolic models, the GSMMs are systematically reduced, and then the kinetic parameters data for the reactions are integrated with the reduced network. Various methods are used to reduce the network, such as

Table 1
A list of some of the classical and recent synthetic biology innovations used to design cyanobacterial strains as workhores for biotechnological applications.

Techniques	Strain	Summary	References
RB-Tnseq*	*Synechococcus* sp. PCC 7942	Identified the essential gene sets	(Rubin et al., 2015)
RB-Tnseq	*Synechococcus* sp. PCC 7942	Identified genes necessary for diurnal growth	(Welkie et al., 2018)
Pooled CRISPRi	*Synechocystis* sp. PCC 6803	Increased L-lactate production	(Yao et al., 2020)
Antisense Expression	*Synechococcus* sp. PCC 7942	Increased production of short chain fatty acids	(Gong and Miao, 2019)
CRISPRi	*Synechocystis* sp. PCC 6803	Identification of an essential gene in ACP (acyl carrier protein)-consumption	(Kaczmarzyk et al., 2018)
Grad-seq	*Synechocystis* sp. PCC 6803	Identification of ribonucleoprotein complexes	(Riediger et al., 2021)
CRISPR/Cpf1	*Synechocystis* sp. PCC 6803	Marker-less gene insertion and deletion	(Ungerer and Pakrasi, 2016)

*RB-Tnseq = Randomly barcoded transposon insertion sequencing.
**RBS = Ribosome-binding sites.

Table 2
Various algorithms applied for the production of target bioproducts through strain designing based on model simulations.

Strain	Algorithm used	Product/ culture condition	Descriptions	Reference
Synechocystis sp. PCC 6803	FBA	n-butanol	8 mg/gDCW/day n-butanol in nitrogen-deprived conditions upon overexpression of heterologous Phosphoketolase (PKT).	(Anfelt et al., 2015)
Synechocystis sp. PCC 6803	FBA	Ethanol	Predicted that ~ 235% (1.054 mmol/gDCW/h) higher ethanol can be produced through 13 genetic manipulations.	(Lasry Testa et al., 2019)
Synechocystis sp. PCC 6803	FVA	Isoprene	40-fold increase in titer/g DCW (2.8 mg/gDCW or 1.0 mg/L).	(Englund et al., 2018)
Nostoc sp. CCC-403	Multi-objective hybrid machine learning	Phycobiliproteins (PBPs)	Identified potential metabolic fluxes contributing to PBPs production.	(Saini et al., 2021, p. 40)
Synechococcus sp. PCC 7942	MCA	Limonene	Increase in productivity by 100-fold (76.3 μg/L/OD/d).	(Wang et al., 2016a)
Synechocystis sp. PCC 6803	MCA	Ethanol	Improved ethanol productivity by 1.37-fold (118.2 ± 8.0 mg/L) by overexpression of phosphoglycerate kinase (PGK).	(Nishiguchi et al., 2019)
Synechococcus elongatus PCC 7942	FBA with MOMA	1,3-propanediol and glycerol	28% improvement in 1,3-PDO and 111% improvement in glycerol titers.	(Hirokawa et al., 2017)

Table 3
The advantages and challenges of different flux analysis approaches.

Approaches	Advantages	Challenges
Structural Models	Predict organism phenotype without the need of extensive experimental work (qualitative estimation of fluxes). Identify fluxes through the whole metabolic network.	Flux distribution depends upon assumptions and constraints. Need validation through experiments.
Kinetic Models	More precise. Can give profiles of multiple intracellular metabolites.	Difficult to get accurate enzyme kinetic data. The system of equations obtained may suffer from instability.
Stationary-MFA	Both qualitative and quantitative estimation of flux values. Easy to estimate fluxes through quantification of amino acids (slower labeling rate and higher abundance than intracellular metabolites).	Can only be applied in mixotrophic conditions. Takes much time (in hours) to reach the steady-state. Difficult to confirm isotopic steady state.
Non-stationary -MFA	Only one Carbon substrate is required. Need less time for labeling experiments.	Fluxes only through central carbon metabolic pathway reactions. Requires rapid quenching of metabolites. More challenging to fit the experimental data.

NetworkReducer (Erdrich et al., 2015), minNW (Röhl and Bockmayr, 2017), etc.

3. Metabolic flux analyses of cyanobacterial metabolism

While the GSMMs provide flux distribution consistent with a particular objective, e.g., growth maximization, under a specific set of condition(s) and constraints, sometimes metabolic fluxes may not follow the assumed objective. The quantitative flux distribution is estimated through stable isotope-based labeling studies called the Metabolic Flux Analysis (MFA) (Zamboni et al., 2009). In isotope labeling experiments (ILEs), the labeled intracellular metabolites/amino acids are estimated using analytical techniques such as mass spectrometry (MS) or nuclear magnetic resonance (NMR) (Antoniewicz et al., 2007; Szyperski, 1995). This labeling pattern is analyzed with specialized software and fitted into a computational model to extract the flux information. MFA can provide valuable information on metabolic mechanisms, regulatory bottlenecks, partitioning of fluxes into diverging pathways at branch points, discovering unusual pathways, identifying the genetic differences in closely related microorganisms, and identifying the potential ways to improve strain performance to eventually maximize product yield (Dai and Locasale, 2017). This approach has been applied to many diverse organisms (including prokaryotes and eukaryotes) cultivated in a variety of physiological conditions (heterotrophy, mixotrophy, starvation, etc.) (Guo et al., 2016).

Two different types of MFA can be performed: steady-state MFA (SS-MFA) and isotopically nonstationary (INST)-MFA (Adebiyi et al., 2015; Cheah and Young, 2018). The SS-MFA approach is mainly applied to heterotrophic or mixotrophic cultures by using ^{13}C labeled glucose, glycerol, or amino acids as a substrate. The term "steady-state'' refers to the metabolic and isotopic steady-states. Both approaches have advantages and challenges (Table 3).

The flux distributions measured by MFA can also be used to validate the predictions, further refine metabolic models, reveal hidden metabolic bottlenecks, and narrow down the range of possible flux distribution measurements provided by FBA (Basler et al., 2018). Several studies have employed INST-MFA to elucidate the differences in metabolic flux distribution in cyanobacterial strains, whether closely-related but showing different phenotypes, or upon genetic engineering (Table 4).

While earlier studies employed INST-MFA to calculate the flux distribution of the central metabolic pathways, recent studies have extended the network to genome-scale metabolic networks. The first such study in photoautotrophs, conducted for *Synechocystis* sp. PCC 6803 (Gopalakrishnan et al., 2018) showed that most of the fixed carbon was used for biomass accumulation while the remaining small percentage moved towards the storage of glycogen and organic acids. Approximately 12% of the fixed carbon was oxidized to CO_2 through the TCA cycle and other anabolic reactions, resulting in a significant loss of carbon. In another study, intracellular flux distribution was recomputed using genome-scale isotopic INST-MFA on two different cyanobacterial strains, *Synechococcus* UTEX 2973 vs. *Synechocystis* sp. PCC 6803 (Hendry et al., 2019). The flux map revealed the differences in the CBB pathway that contribute to more efficient carbon metabolism and thus higher growth in *Synechococcus elongatus* UTEX 2973. More applications of combining ^{13}C-MFA with GSMMs can be expected in the near future.

4. Metabolomics

Cyanobacteria produce a wide variety of unique active secondary metabolites, which are gaining attention due to their anti-bacterial, anti-inflammatory, anti-cancer properties (Jeong et al., 2020). CyanoMetDB is a public repository for secondary metabolites of cyanobacteria (Jones et al., 2021). Improvements in the sensitivity and resolution of analytical techniques, along with development of data analyses tools, have facilitated the identification of a large number of metabolites. For example, a novel sequential window acquisition of all theoretical fragment-ion spectra (SWATH) approach quantifies untargeted precursors and MIDs of intracellular metabolites (even at low abundance) from a ^{13}C-labeled experiment in a single run with greater resolution and fewer errors than conventional techniques (Jaiswal et al., 2018a).

Table 4
Various applications of INST-MFA approach on cyanobacteria.

Base Strain	Conditions compared	Major Finding(s)	Reference
Synechococcus sp. PCC 7002	Wild-type (WT) and glycogen synthase (*glgA*) gene knockout	Flexible carbon partitioning between ADPG and UDPG, less carbon flux towards G1P and a marginal increase towards glycolysis and the TCA cycle are associated with increased glucosylglycerol and sucrose production by the mutant.	(Hendry et al., 2017)
Synechococcus elongatus strains (nearly identical at genome level)	Compared UTEX 2973 and PCC 7942 strains	Even though the genome sequence of UTEX 2973 is nearly identical to PCC 7942, the faster growth is due to higher flux through the CBB cycle, glycolysis, and pyruvate kinase pathway, but limited flux in the TCA cycle and negligible in malic enzyme and Oxidative-PPP (OPPP).	(Abernathy et al., 2017)
Synechococcus sp. PCC 7942	WT and isobutyraldehyde (IBA)-producing strains	Identified a possible puruvate kinase (PK) bypass pathway and the existence of malate dehydrogenase (MDH) activity in both strains.	(Jazmin et al., 2017)
Synechococcus sp. PCC 7002	WT and mutant (ΔccmKLMN) strain lacking carboxysome	The mutant could recover its growth by achieving asimilar metabolic flux distribution as that of WT in spite of reduced photosynthesis and higher biomass accumulation, protein content, and photorespiration activity.	(Abernathy et al., 2019)
Synechococcus sp. PCC 7002	Nitrogen replete and deprived conditions	The flux through the bottleneck reaction for glycogen synthesis under nitrogen depleted conditions, as well as increased flux overflows through the hybrid gluconeogenesis–pentose phosphate (hGPP) pathway.	(Qian et al., 2018)
Synechocystis sp. PCC 6803	WT and ΔnrtABCD mutant in Nitrogen limited condition	The mutant had higher flux values for glycogen synthesis, OPPP and anaplerotic pathways and a waste cycle for ATP consumption to acclimatize in nitrate-limited conditions.	(Nakajima et al., 2017)

The metabolomics analyses have revealed several novel insights into cyanobacterial biology. For example, it was shown that *Synechococcus elongatus* UTEX 2973 fixed more carbon through the pentose phosphate pathway (PPP) and redirected its flux to synthesize storage compounds from the glycolytic pathway for enhancing salt tolerance (Cui et al., 2020). In another study, it was revealed that UV-B exposure significantly reduced intracellular metabolites related to carbon and nitrogen metabolism, such as amino acids, which play a role in secondary metabolite production and significantly increased the accumulation of the cyto-protective amino acid proline, in *Chlorogloeopsis fritschii* cells (Kultschar et al., 2019). Another study on *Synechococcus elongatus* PCC 11801 showed that the cellular metabolite inventory undergoes dramatic changes during diurnal growth, some of which changed up to 100-fold. The intermediate of the CBB cycle peaked during mid-day for faster growth. The unknown gamma-glutamyl dipeptides (which act as amino acid reservoirs) and several other storage molecules or their precursors were accumulated in the cells during the dark period (Jaiswal and Wangikar, 2020). A comparative analysis estimating the metabolites of *Synechococcus elongatus* PCC 11802 and *Synechococcus elongatus* PCC 11801 revealed that *Synechococcus elongatus* PCC 11802 has relatively more active reactions involved in CO_2 assimilation catalyzed by RuBisCO and PEPC and the presence of additional intermediates of the CBB cycle with minor triose phosphate utilization (Jaiswal et al., 2020). Similarly, comparative metabolomics analysis of a library of 44 response regulators (RRs) knockout strains of *Synechocystis* sp. PCC 6803 indicated their differential regulatory roles (Shi et al., 2020).

5. Transcriptomics

To precisely determine the metabolic state of a cell, the dynamics of changing transcript pools are required. Additionally, the obtained information can be added to the GSMMs to further constrain the solution space of the model. The transcriptomic data of a large variety of cyanobacterial strains can be accessed online through publicly available databases such as CyanoEXpress (Hernandez-Prieto and Futschik, 2012).

The study conducted by Pei et al. (2017) identified 133 *trans*-encoded sRNAs (small RNAs), among which 23 sRNAs were identified for the first time in *Synechocystis* sp. PCC 6803. The overexpression of one of the identified sRNAs (Nc117) improved the tolerance against ethanol and butanol in *Synechocystis* sp. PCC 6803 (Pei et al., 2017). Recently, the RNA-seq analysis of an alkane producing strain of the filamentous cyanobacterium *Nostoc punctiformae* PCC 73102 revealed the potential negative regulators of alkane production (Arias et al., 2020). Such studies provide a basis for the engineering of cyanobacteria for the production of valuable bioproducts.

The supplementation of RNA-Seq with other tools such as RNA polymerase (RNA pol), ChIP-sequencing, and Grad-seq has further enhanced the capabilities of transcriptomic analyses by unraveling protein-RNA interactions (Riediger et al., 2021). Recent advancements in transcriptomic tools have highlighted the previously undetermined roles of sRNAs, asRNAs (antisense RNAs), and some riboswitches in cyanobacterial metabolism (Mironov et al., 2021; Till et al., 2020). Therefore, these ncRNAs could be considered for designing methods and metabolic engineering protocols to optimize metabolic flux distribution in cyanobacteria (Muro-Pastor and Hess, 2020). Though the potential of engineering ncRNA expression is yet to be realized in cyanobacteria, the capability of the information obtained from transcriptomics datasets has been much appreciated in designing targeted engineering experiments in cyanobacteria. For instance, transcriptomic analyses revealed the responsive genes (mainly encoding ROS-degrading proteins and potential transporters of free fatty acids or FFA) involved in mitigating oxidative stress caused by the biosynthesis of FFA in *Synechococcus* sp. PCC 7942 (Ruffing, 2014, 2013). The responsive genes were targeted for mutagenesis to confirm reduced toxic effects and improved FFA yield in cells. A recent transcriptomics study has revealed a significant role of asRNAs in *Synechocystis* sp. PCC 6803 cells when exposed to hexan-1-ol stress (Mironov et al., 2021). Further, the integrative approaches are proving to be efficient in unraveling the unique metabolic properties of cyanobacteria. For example, the integration of RNA-seq with a methodology for cataloging the genome architecture (differential RNA-seq or dRNA-seq) and identifying 3′-end positions and 3′-untranslated regions in transcripts (Term-seq) led to the identification of a distinctive energy generation strategy in *Synechocystis* sp. PCC 7338 (Jeong et al., 2021).

The emergence of advanced transcriptomic techniques, such as single-cell transcriptomic (scRNA-Seq), is promising to tackle population heterogeneity-related problems (Srivastava and Shukla, 2021). For example, industrial-scale photobioreactors are likely to generate a gradient in pH, substrate, temperature, or dissolved carbon concentration due to their large size and non-uniform mixing conditions (Nadal-Rey et al., 2021b). These local environments generate locally adapted

subpopulations that differ in their metabolic states. Integration of single-cell sorting methods (Fluorescence-activated Cell Sorting) and scRNA-Seq could be a promising approach in unraveling the target proteins that can be further engineered to design stable and superior strains that are less affected by local environmental changes (Nadal-Rey et al., 2021a).

6. Proteomics analysis: tools, techniques, and challenges

Proteomics represents a powerful approach to investigate the basis of metabolome changes guided by protein expression and their post-translational modifications in cyanobacterial cells (Srivastava and Shukla, 2021). The proteomic changes in cyanobacteria have been systematically studied by sub-proteome analysis of the thylakoid membrane and cytoplasm. While the cytoplasmic proteome provides a global snapshot of cellular changes in the cells, the thylakoid proteome exclusively allows the study of biogenesis, assembly, and dynamics of photosynthetic apparatuses (Shi et al., 2021). Besides these fractions, the analysis of the exoproteome is one of the emerging branches of proteomics that specifically analyzes the secreted proteins in the medium (Flores and Tamagnini, 2019). So far, the most common method for quantitative proteomics of cyanobacteria is iTRAQ (Isobaric tags for relative and absolute quantitation) (Chang et al., 2017). This technique utilizes isobaric reagents that can effectively bind to the primary amine group of proteins and peptides.

The metabolome of cyanobacteria is regulated not only by amounts of proteins (enzymes) but also by a variety of post-translational modifications (PTMs) such as phosphorylation (phosphatase and kinase activity), acetylation (Babele et al., 2019; Xiong et al., 2016) and so on. The PTMs play a crucial role in the signal transduction and regulatory pathways in cyanobacteria. In particular, acetylation of proteins regulates the protein stability, enzymatic activity, protein interaction, and cellular localization of proteins and metabolites inside the cells. Therefore, the proteome analysis of these modifications has emerged as important sub-branches of proteomics named phosphoproteome and acetylome (Mo et al., 2015; Spät et al., 2021). A phosphoproteomic analysis of *Synechococcus* sp. PCC 7002 found 410 phosphorylation sites on phosphoproteins participating in crucial molecular methods such as two-component signal transduction pathways and photosynthesis (Yang et al., 2013). The identification of Serine/Threonine/Tyrosine kinases and phosphatases further plays a central role in controlling carbon/nitrogen metabolism in cyanobacteria. Such information might be beneficial while designing metabolic engineering efforts to redirect the metabolic flux towards a target bioproduct in cyanobacteria. The global analysis of the lysine acetylome in *Synechocystis* sp. PCC 6803 found 776 acetylation sites (Mo et al., 2015). Most of the acetylated proteins that appeared were involved in cellular metabolism, including the phycobilisome subunits. Further, analysis of lysine malonylation (addition of a malonyl group) identified 598 sites that were found to be involved in photosynthetic reactions (Ma et al., 2017). These molecular clues on the PTMs of amino acid residues will offer a novel vision into the regulation of photosynthesis and other metabolic processes that can be utilized for the construction of metabolically superior strains of cyanobacteria.

7. Integrated omics

The DBTL cycle, even though an organized and effective approach for strain design, requires high quality and multi-omics datasets to increase the precision and robustness of learning. Since an organism's genome-to-phenotype link is still not fully understood, cell phenotypes can differ at various levels, even for species with nearly identical genomes. Thus, the attention is turning towards utilizing and combining different omics technologies to facilitate a more comprehensive understanding of cell physiology and the metabolic capabilities of biological processes (Amer and Baidoo, 2021). The omics approaches have been applied successfully in many fields to understand cellular behavior, symbiotic associations, wastewater treatment, metal toxicity, biofuel production, pharmaceutical and therapeutics, cosmeceuticals, and to enhance the productivity of target biochemicals (Lin et al., 2019; Mishra et al., 2019). The multi-omics studies information is integrated with the system biology approach for developing cyanobacteria as a host for biotechnological applications (Fig. 2).

Recently, multi-omics data are becoming publicly available, and it is believed that GSMMs are promising scaffolds to use these datasets. CyanOmics is a database for integrated omics of *Synechococcus* sp. PCC 7002 containing information about the complete genome sequence with functional annotation, metabolomics, transcriptomes, and proteomic analyses under variable conditions (Yang et al., 2015). The GSMMs reconstructions are converted into predictive models by incorporating transcriptional regulatory connections and high-throughput omics data as constraints to obtain context-based models (CBM) (Zhou et al., 2021). Two significant expansions in GSMMs combined with protein information are GEM-PRO (genome-scale models with protein structures) and ME models (Models integrating metabolism with protein expression). GEM-PRO permits structural bioinformatics analysis from a systems-level perspective (Brunk et al., 2016). Recent expansions in network content with expression data result in the production of ME-Models, but the process needs very detailed information (O'Brien and Palsson, 2015). Several attempts have been made to integrate datasets from a given experiment to obtain a CBM variant and decrease the solution space by adding constraints through the integration of omics data. Advances in omics approaches and bioinformatics tools and databases will play an essential role in achieving economically feasible targets. More data from diverse cyanobacterial species may unravel novel, universal regulatory principles.

With the increasing importance of multi-omics data, several tools, databases, algorithms, and methods (e.g., GECKO (GEMs with Enzymatic Constraints using Kinetic and Omics data) (Sánchez et al., 2017), ME, REMI (Relative Expression and Metabolomics Integrations) (Pandey et al., 2019), etc.) have been developed to incorporate omics data with metabolic models with advanced statistics such as principal compound analysis, multivariate data analysis, etc. and data depictions through correlation maps, volcano plots, etc. (Bekiaris and Klamt, 2020; Zampieri et al., 2019). Various algorithms such as GIMME (Gene Inactivity Moderated by Metabolism and Expression) (Becker et al., 2008), iMAT (the Integrative Metabolic Analysis Tool) (Shlomi et al., 2008), MADE (Metabolic Adjustment by Differential Expression) (Jensen and Papin, 2011), IOMA (Integrative Omics-Metabolic Analysis) (Yizhak et al., 2010), etc. are used for the integration of metabolomics and expression data with CBM (Volkova et al., 2020). The DeepRiPP database is used for integrating genomics and metabolomics data to find out novel ribosomally synthesized posttranslationally modified peptides (RiPPs) (Merwin et al., 2020). TREM-Flux (Time-Resolved Expression and Metabolite-based prediction of flux values) incorporates partial time-resolved unlabeled metabolomics and transcriptomic data with CBM (Kleessen et al., 2015) to predict non-steady-state fluxes in response to a perturbation, such as a treatment or a change of environmental conditions. These techniques are constructed on constraint-specific formulations, and the reduction of metabolic models needs to be optimized further to study these added constraints.

The proteomic and metabolomics approaches were applied to the 3-hydroxypropionate-(3-HP)-producing *Synechocystis* sp. PCC 6803 strain (Wang et al., 2016b). The results concluded numerous characteristics of cellular metabolism related to energy, reducing equivalents, basic metabolism and biomass compound synthesis, which were differentially enhanced in the 3-HP-producing strain. A systems analysis based on omics data conducted on *Synechococcus* sp. PCC 7002 for enhanced ethanol production (Kopka et al., 2017) showed that during ethanol production, the intermediates of pyruvate-based pathways were decreased due to unnecessary carbon loss from the primary metabolism. Oftadeh et al. (2021) used the ETFL/yETFL (Expression and Thermodynamics FLux) method to efficiently integrate RNA and protein

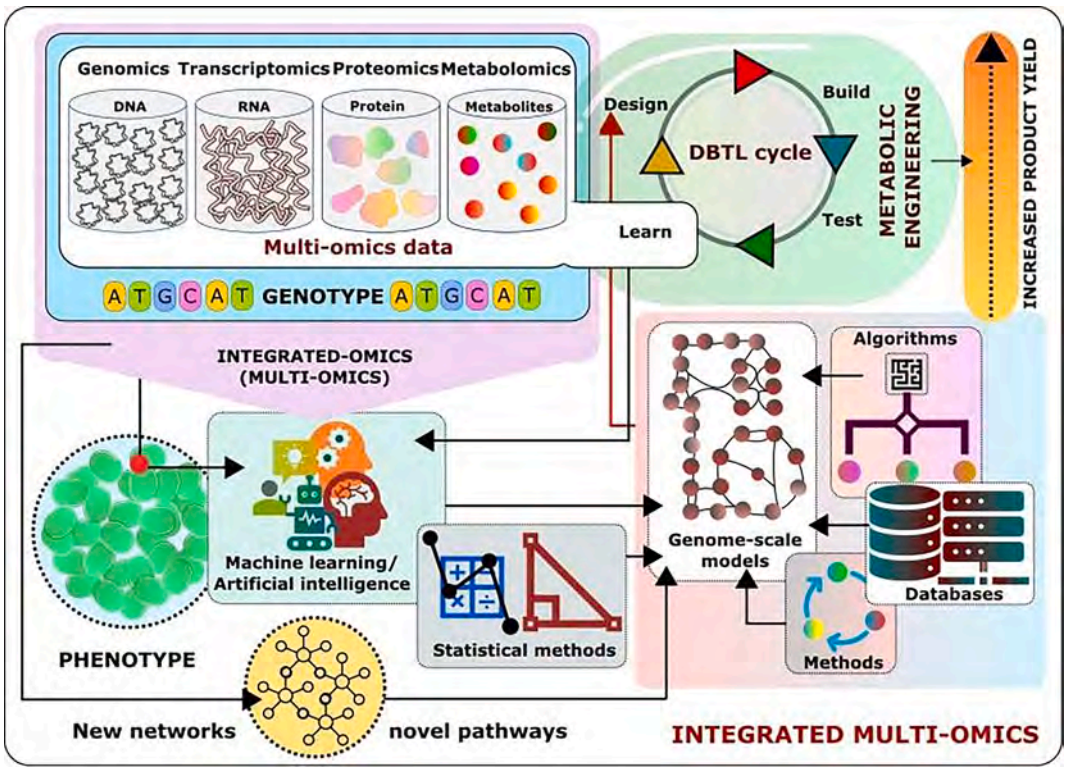

Fig. 2. Illustration of the multi- and integrated-omic approachs for understanding the cellular metabolism for strain engineering purposes. The integration of omics data requires more additional constraints from genomics, metabolomics, transcriptomic, proteomics and fluxomics techniques for further model refinement and predictions.

synthesis data with GSMM to predict maximum growth rate, essential genes, and the phenotype of overflow metabolism, which provides valuable visions on the optimality of the regulatory mechanisms and can be further used to design strategies to make valuable strains (Oftadeh et al., 2021). In another study, integration of transcriptomics and exo-metabolomics with GSMM was done to study dynamic population behavior and changes during adaptation (Hadadi et al., 2020).

8. Conclusions

GSMMs and different Omics analyses have revealed novel insights into cyanobacterial biology and regulation. However, understanding the dynamic activities of complex metabolism is required to predict cellular behavior through systems and synthetic biology tools. The integration of high-throughput omics has emerged as a beneficial approach to provide in-depth information about metabolism and its regulations for rational strain designing. In the future, artificial intelligence and machine learning (AI/ML) approaches coupled with omics datasets will generate novel information on regulation at various levels to devise molecular biology-based strategies to precisely and rapidly create designer strains for biotechnological applications.

CRediT authorship contribution statement

Ruchi Pathania: Writing – original draft. **Amit Srivastava:** Conceptualization, Writing – original draft. **Shireesh Srivastava:** Conceptualization, Writing – review & editing, Supervision. **Pratyoosh Shukla:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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