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Review

Advances in the dynamic control of metabolic pathways in *Saccharomyces cerevisiae*

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ABSTRACT

The metabolic engineering of *Saccharomyces cerevisiae* has great potential for enhancing the production of high-value chemicals and recombinant proteins. Recent studies have demonstrated the effectiveness of dynamic regulation as a strategy for optimizing metabolic flux and improving production efficiency. In this review, we provide an overview of recent advancements in the dynamic regulation of *S. cerevisiae* metabolism. Here, we focused on the successful utilization of transcription factor (TF)-based biosensors within the dynamic regulatory network of *S. cerevisiae*. These biosensors are responsive to a wide range of endogenous and exogenous signals, including chemical inducers, light, temperature, cell density, intracellular metabolites, and stress. Additionally, we explored the potential of omics tools for the discovery of novel responsive promoters and their roles in fine-tuning metabolic networks. We also provide an outlook on the development trends in this field.

1. Introduction

Saccharomyces cerevisiae is widely used in the food industry and in the production of various compounds such as biopharmaceuticals, biofuels, and natural active substances because of its easy cultivation, operation, clear genetic background, and accompanying molecular tools [1,2]. *Saccharomyces cerevisiae* can be optimized to enhance its production efficiency through metabolic engineering [3]. A classic method (static regulation) in metabolic engineering involves directing the metabolic flux toward the products of interest by overexpressing or deleting key genes in the metabolic pathway. Through this classic approach, several studies have successfully increased the production of high-value-added natural products, including isoprenoid β-carotene, 3-hydroxypropionic acid, and others [4–7]. However, altering the expression of key genes through a single reduction or increase can have irreversible effects on cell growth. Cellular stress can arise due to imbalanced cofactors or feedback inhibition caused by the accumulation of intermediates [8]. In contrast to static regulation, dynamic regulation has been a widely adopted strategy in nature. From the perspective of metabolic engineering, the implementation of dynamic control allows organisms to adapt their metabolic states to the changing intracellular or environmental conditions in real time. This approach ensures that the cells maintain an optimal production state throughout all stages of culture, thereby enhancing the biological robustness of the host and increasing productivity.

There are two common approaches to dynamic regulation in metabolic engineering [9]. The first approach involves a regulatory system reliant on externally induced signals. During fermentation, chemical inducers or adjustments in physical parameters (such as light, temperature, and pH) can activate or inactivate various transcription factors (TFs). This enables the regulation of gene expression, allows for the control of cell growth and production states, and ultimately optimizes cellular productivity [10,11]. The second approach involves a self-inducible regulatory system that initiates gene expression without relying on external signals as an input. Instead, they dynamically control gene expression by utilizing variations in intracellular metabolite concentrations, cellular stress levels, and cell density. The design of self-inducible gene circuits mainly focuses on balancing the metabolic flux between cell growth and product production, with critical nodes located at the crossroads between cell metabolic and production pathways. By constructing gene circuits related to intermediate metabolites, the rates of synthesis and consumption of these metabolites can be regulated. This regulation helps to achieve a balanced metabolic flux throughout the pathway, thereby promoting product biosynthesis [12,13]. Owing to the limited specificity of current sensing elements, further improvements are required in order to use them in gene circuits [9]. Identifying additional elements, such as TFs or RNA aptamers capable of sensing intermediates or products in metabolic pathways, is essential for achieving effective dynamic regulation [14,15]. Researchers have explored various approaches to achieve dynamic regulation of genes, including the identi-

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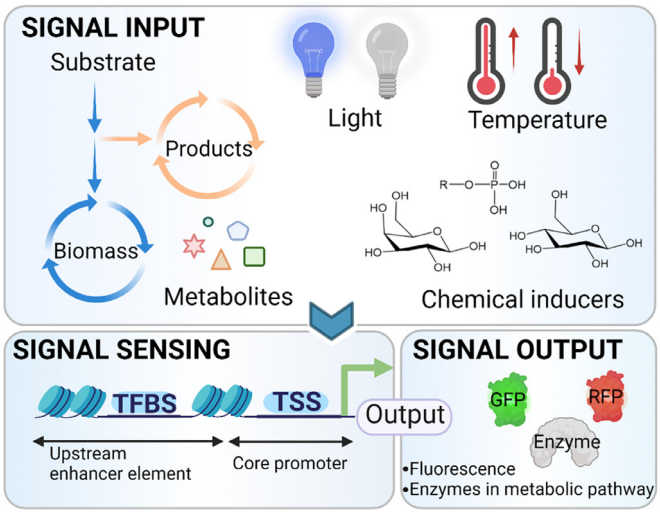

Fig. 1. Components and responses of a biosensor based on a responsive transcription factor and promoter system [22,27]. The ligand-binding domain of a transcription factor specifically detects input signals, resulting in a conformational change of the DNA-binding domain. The interaction between transcription factor (TF) and TF binding site (TFBS) regulates the transcription strength, which is reflected by output signals such as reporter proteins or enzymes in metabolic pathways.

fication of response-repressive elements and the combination of sensor-activated elements with CRISPR/dCas9, RNAi, protein degradation tags, among others [16–18]. These approaches enable the redirection and coordinated distribution of metabolic flux at the transcriptional, translational, and protein levels. Different methods have varying efficiencies depending on the host cell, and the choice of a specific method should be determined based on the intended application.

Presently, dynamic regulation strategies primarily focus on the discovery and application of transcription factors-promoters that respond to metabolites, thus enabling the construction of regulatory systems [19,20]. By targeting the early stages of gene expression and dynamically regulating transcription levels, gene expression can be controlled more rapidly. Transcriptional regulation is effective in simultaneously regulating the levels of multiple RNAs and proteins, making it suitable for simultaneously regulating the expression of multiple genes [15,21].

A transcriptional biosensor comprises three modules: signal input, sensing, and signal output (Fig. 1) [22]. The ligand-binding domain (LBD) of a sensing TF specifically detects input signals such as metabolites and chemical inducers, leading to a conformational change in its DNA-binding domain (DBD). Thereafter, conformationally altered TFs bind to the TF binding sites (TFBs) on the promoter, initiating transcriptional expression of the output signal [23,24]. Transcription factor-based biosensors have emerged as powerful tools in synthetic biology and metabolic engineering. These biosensors can be used for various applications, including strain evaluation, high-throughput screening, and heterologous pathway optimization [23,25]. The construction of biosensors and their role in dynamic regulation has been summarized previously [26,11].

In this review, we focused on the recent developments in dynamic regulatory tools for metabolic pathway engineering in *S. cerevisiae*. These tools include biosensors based on transcription factors (TFs) which are responsive to both endogenous and exogenous signals. Additionally, we explored the potential application of omics approaches for the identification and exploration of response elements.

2. Dynamic regulation based on exogenous signals

Environmental changes can affect the physiological state of cells and provide useful signals for dynamically regulating gene expression. Promoters that respond to exogenous signals can be used to regulate the strength of gene expression or switch synthetic pathways. In the field of metabolic engineering, the dynamic control of gene expression in *S. cerevisiae* often relies on utilizing exogenous signals, including chemical inducers, temperature, and light. Table 1 provides an overview of the recent metabolic engineering studies on *S. cerevisiae* that have employed elements that respond to external signals.

2.1. Chemical-responsive dynamic regulation

Metabolic engineering aims to improve the efficiency of target compound production in cells. However, maximizing the flux within the biosynthetic pathway without considering essential cellular requirements can adversely affect both cell growth and product yield. Dynamic regulation is an effective strategy for balancing the two stages of cell growth and product synthesis [10]. When biomass accumulation reaches a certain threshold and must be switched to product biosynthesis, the metabolic flux to the biosynthetic pathway can be increased by adding chemical inducers to achieve a balance between cell growth and product synthesis [45]. Carbon sources, nutrients, and ions serve as chemical induction signals. Supplementation with these chemicals enables yeast cells to modulate the transcription of genes, leading to their upregulation or downregulation. For example, glucose as a carbon source can induce the expression of promoters, such as P_{HXT1} and P_{HXT3}, and yeast cells use glucose for rapid growth [46,47]. Maury et al. [28] identified and characterized glucose-dependent promoters as dynamic control elements; one such promoter, P_{ICL1}, was used to regulate 3-hydroxypropionic acid (3HP) production based on glucose concentration. In the presence of excess glucose, P_{ICL1} inhibits 3HP synthesis. Conversely, under glucose-limited conditions, the activation of 3HP synthesis was achieved through P_{ICL1}, leading to a 70% increase in 3HP production compared to the P_{PGK1} promoter-based regulation of the 3HP pathway. Bian et al. [29] developed a dual-signal hierarchical dynamic regulation system that responded to both glucose concentration and culture temperature, thereby dividing lutein biosynthesis into three stages. Genes responsible for delta-carotene formation were placed under the glucose-responsive *ADH2* promoter, whereas genes involved in the conversion of delta-carotene to lutein were placed under the temperature-responsive *GAL* promoter. This strategy effectively resolves the conflict between growth and production and mitigates intra-pathway competition concerns. The galactose-induced GAL system exhibits high expression intensity and has been used for the biosynthesis of valencene [30], polyketides [31], and D-limonene [32]. However, galactose is not the preferred carbon source for yeast growth, and the presence of glucose in the medium inhibits expression of the GAL system. To address this, a glucose-responsive GAL regulation system was developed for dynamic regulation in yeast through *GAL80* knockout [48,49]. During the early stages of fermentation, a significant portion of the carbon source is allocated for supporting rapid cell growth. As the glucose supply is depleted, genes within the engineered biosynthetic pathway, regulated by the P_{GAL} promoter, are upregulated to facilitate the accumulation of desired products, such as hydroxytyrosol, ethanol, and methyl ketones [50,51].

In addition to carbon sources, inorganic phosphates, copper ions, and amino acids can serve as inducers in the dynamic regulation of pathways in *S. cerevisiae* [52]. Teixeira et al. [33] used a copper-induced P_{CUP1} promoter to dynamically regulate the expression of fatty acyl-CoA synthase Faa1 and adjust the conversion of free fatty acids to fatty acyl-CoA. This approach avoids the excessive release of free fatty acids and waste of precursor substances when the fatty acyl-CoA synthases Faa1 and Faa4 are directly deleted. In a recent study, Zhou et al. [34] leveraged the benefits of the native GAL system and copper-responsive promoters to create a novel copper-inducible GAL regulation system (CuIGR). Specifically, they used the copper-inducible *CUP1* promoter to regulate the expression of Gal4, while the copper-repressed *CTR3* promoter controlled Gal80 expression. This system facilitated the dynamic control of

Table 1
Application of exogenous signal-responsive elements in metabolic engineering of *S. cerevisiae*.

Signal type	Signal input	Response element	Product	Result	Refs.
Chemical	Glucose	P_{ICL1}	3-hydroxypropionic acid	1.70-fold	[28]
	Glucose	P_{ADH2}	lutein	19.92 mg/L	[29]
	Galactose	P_{GAL}	valencene	539.3 mg/L	[30]
	Galactose	P_{GAL}	bikaverin	202.75 mg/L	[31]
	Galactose	P_{GAL}	D-limonene	6.28 mg/L	[32]
	Cu^{2+}	P_{CUP1}	fatty alcohol	1.41-fold	[33]
	Cu^{2+}	P_{CUP1}, P_{CTR3}	lycopene	33-fold	[34]
	Phosphate	P_{PHO5}	xylose reductase	–	[35]
	Methionine	P_{MET3}	fragrant terpenoids	101.7 mg/L	[36]
Light	Blue light	LVAD, $LexA_{op}$	mCherry	500-fold	[37]
	Blue light	OptoEXP, OptoINVRT	isobutanol; 2-methyl-1-butanol	8.49 g/L; 2.38 g/L	[38]
	Blue light	OptoAMP	lactic acid, isobutanol, naringenin	6.0 g/L 830 mg/L 1.76-fold	[39]
	Blue light	OptoQ-AMP OptoQ-INVRT	acetoin	35 g/L	[40]
Temperature	Temperature	Gal4M9	lycopene	2.77-fold	[41]
	Temperature	Gal4M9	astaxanthin	235 mg/L	[42]
	Temperature	Gal4M9	tocotrienols	320 mg/L	[43]
	Temperature	Gal4M9	crocetin	139.67 μg/g-DCW	[44]

gene expression under the regulation of $P_{GAL1/2/7/10}$ promoters, utilizing cost-effective $CuSO_4$ as the effector. Furthermore, integrating a degradation tag at the N-terminus of Gal80 expanded the dynamic range of the CuIGR system. Consequently, the engineered CuIGR4 system exhibited an enhanced response to copper by up to 2.7-fold, resulting in a remarkable 72-fold induction of EGFP expression and a 33-fold increase in lycopene production, compared to the native Cu^{2+}-inducible *CUP1* promoter [34]. The promoter P_{PHO5} is repressed in a high-phosphate medium and de-repressed in a low-phosphate medium [53] and has been used to regulate the expression of recombinant xylose reductase when displayed on the yeast surface [35]. Additionally, the methionine-repressed promoter P_{MET3} was used to regulate the expression of squalene synthase Erg9. This approach effectively reduces the metabolic flux of the competing pathway, leading to an increased production of fragrant terpenoids [36].

By using different chemical inducers, it is possible to achieve dynamic regulation of multiple genes, thereby avoiding the potential mutual influence between the regulation of different genes that may arise when using a single inducer. However, additional investigation and optimization are necessary to ensure an appropriate dosage of the inducer, considering the state of the yeast strain and to prevent the expression leakage effect caused by the analogs present in the nutrient-rich medium. Recently, computer-based automatic control algorithms have been developed to assist in dynamic regulation. A promising approach to achieve the dynamic regulation of gene expression is the use of mathematical models to simulate the dynamic interplay between gene expression and metabolism [45]. Another approach involves integrating flow cytometry or microfluidic devices with fluorescent reporters and computer feedback control algorithms, enabling continuous, rapid, and controllable regulation [54,55]. These approaches show great potential for achieving dynamic regulation of gene expression.

2.2. Light-responsive dynamic regulation

Although the use of chemicals as inducers is common, it is important to consider that certain chemicals can be expensive and potentially interfere with metabolic pathways. Furthermore, reversal of this induction may pose challenges. Light- and temperature-induced systems are promising alternatives that address these limitations [56]. As an induction signal, light offers several advantages of low cost, minimal impact on cells, spatiotemporal regulation, and reversibility [57,58]. Optogenetic systems respond to various wavelengths. Previous studies have successfully combined different light-responsive systems to achieve orthogonal regulation by reducing interference between optogenetic systems, thereby enabling more precise and reliable regulation of gene expression [59,60].

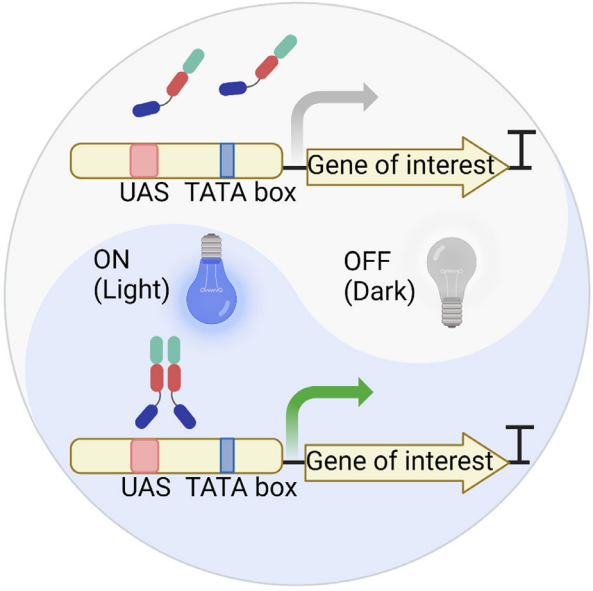

Fig. 2. Light-responsive regulation system [61]. After being activated by light, the photoreceptor protein usually undergoes homodimerization and interacts with upstream activation sequences (UAS) to initiate gene expression.

Photoreceptor proteins are induced by specific wavelengths of light. After light exposure, photoreceptor proteins undergo conformational changes to form oligomers or polymers. This system relies on light-induced transcription factors and consists of a DNA-binding domain (DBD) and a light-responsive domain (VVD). Upon exposure to blue light, light-inducible TFs undergo dimerization and subsequently bind to upstream activation sequences (UAS) to directly inhibit or activate gene transcription [61] (Fig. 2). Optogenetic tools can adjust the expression

level and duration of key genes by controlling the intensity and duration of light exposure [62,63].

The optogenetic system was first used in neuroscience research to enable the fast and simple optical control of neurons [64]. However, the applications of complex light-inducible expression systems are limited. Xu et al. [37] developed a single component optogenetic system, called the yLightOn system, to control gene expression in yeast cells. This system comprises a LexA-VVD light-switchable TF fused with a Gal4 activation domain (LVAD) that controls the expression of red fluorescent protein (mCherry) in response to light signals. The yLightOn system is simple and tightly regulated, with an ON/OFF expression ratio of over 500. This system utilizes the repressor protein LexA, which is derived from the SOS response system of *Escherichia coli*. Therefore, it was orthogonal to the genetic molecular elements of yeast cells, which reduced leaky expression by avoiding crosstalk with yeast cellular components. The use of an ssrA protein degradation tag-based light-controlled bidirectional expression module enables the spatiotemporal control of protein stability in yeast cells [37].

Zhao et al. [38] developed two optogenetic circuits, OptoEXP and OptoINVRT, to control yeast cell growth and increase the production of valuable products. OptoEXP is a light-inducible optogenetic circuit, whereas OptoINVRT is a light-repressible circuit. Using these circuits, the production levels of isobutanol and 2-methyl-1-butanol reached 8.49 g/L and 2.38 g/L, respectively [38]. However, the use of light-inducible regulatory systems in large-scale fermentation is hindered by the limited light penetration in bioreactors. To overcome this issue, blue light-activated OptoAMP circuits were constructed to increase the transcriptional response to light [39] based on the previously constructed optogenetic circuit OptoEXP and the GAL regulon system. OptoAMP enhanced the production of lactic acid, isobutanol, and naringenin via light-controlled three-phase fermentation (growth, induction, and production phases).

This optogenetic system has limited ability to simultaneously activate and suppress genes, as both the suppression (OptoINVRT) and activation (OptoAMP) circuits are based on the GAL regulon system. Therefore, Lalwani et al. [40] introduced the Q System, a transcriptional regulation system from *Neurospora crassa*, to construct new light-inducible circuits (OptoQ-AMP) and light-repressible circuits (OptoQ-INVRT). As the Q and GAL regulon systems are orthogonal, new optogenetic tools (OptoQ-AMP and OptoQ-INVRT) can be used in combination with the previous system to achieve synchronous activation and suppression of different genes under light control. The utilization of two orthogonal optogenetic circuits (OptoQ-AMP and OptoQ-INVRT) to regulate multiple genes in the acetoin synthesis pathway resulted in a substantial increase in the acetoin yield, reaching 35 g/L [40].

2.3. Temperature-responsive dynamic regulation

Temperature is a critical control parameter in cell culture that can significantly affect the fermentation process. Gene expression can be regulated by temperature, even during the late cell growth phase. Wang et al. used temperature-induced control systems to achieve the bidirectional regulation of gene expression at high cell densities (OD_{600}≈50), demonstrating the potential of temperature control for scaling up microbial fermentation [58].

Temperature regulation involves controlling temperature to influence the binding of temperature-sensitive proteins to promoters, thereby regulating gene expression. Several temperature-inducible expression elements or systems have been reported in yeast, including the temperature-sensitive acid phosphatase promoter [65], the MATα system inhibited by the temperature-sensitive protein Sir3 [66], and temperature-sensitive variants of the Gal4 TF [41,67]. Among these, the GAL system has the advantages of being easy to operate and inducing high levels of heterologous gene expression [68,69].

The Snf1-Mig1 pathway represses the expression of *GAL4* at high glucose concentrations, thereby inhibiting the activity of the P_{GAL} promoter. In the absence of galactose, the regulatory factor Gal80 binds to the transcriptional activator Gal4, thereby preventing the initiation of P_{GAL} transcription. However, under glucose-limiting conditions and in the presence of galactose, Gal3 binds to Gal80, inducing a conformational change that releases Gal4, and subsequently activates the transcription of P_{GAL} promoters [70]. Negative regulation of the GAL system by intermediate metabolites in response to glucose can result in the incomplete prevention of gene expression at low glucose concentrations, leading to the leakage of expression during the growth phase. To address this issue, Zhou et al. [41] constructed a temperature-dependent GAL system by deleting Gal80 and introducing the evolutionarily temperature-sensitive Gal4 mutant, Gal4M9. After verifying the temperature-responsive induction ability of the system using enhanced green fluorescent protein (eGFP), it was applied to regulate the biosynthesis of lycopene, resulting in a 44% increase in cell biomass and a 177% increase in lycopene production. These findings provide a new approach for controlling the biosynthesis of value-added compounds.

Accumulation of astaxanthin often hinders cell growth, posing a challenge for achieving high-density fermentation. Zhou et al. [42] overcame the metabolic bottleneck in the astaxanthin biosynthesis pathway by directing the co-evolution of β-carotene hydroxylase and β-carotene ketolase to accelerate the conversion of β-carotene to astaxanthin. Subsequently, a temperature-responsive regulation system based on Gal4M9 was introduced to decouple astaxanthin production from cell growth, resulting in the production of 235 mg/L astaxanthin through two-stage, high-density fermentation. Shen et al. [43] designed a cold-shock temperature control system using the temperature-sensitive mutant, Gal4M9, to regulate cell growth and tocotrienol accumulation during high-density fermentation. With the implementation of this two-stage fermentation process, the engineered strain produced of 320 mg/L tocotrienols.

Moreover, temperature-responsive regulation systems can be used to study the temperature requirements for the activities of key enzymes in metabolic pathways. For example, in the biosynthesis pathway of crocin, the substrate zeaxanthin can be effectively generated and stored in the cell membrane at 30 °C. However, the activity of Ccd2, which is located in the cytoplasm, is significantly enhanced at low temperatures alone, limiting the yield of crocin. The Gal4M9 temperature regulation system enabled synchronous biosynthesis and conversion at 25 °C, greatly improving the efficiency of zeaxanthin conversion to crocin and resulting in a final crocin yield of 139.67 μg/g DCW [44]. These studies demonstrated the potential of the Gal4M9 temperature regulation system in increasing the production of carotenoid derivatives and highlighted the advantages of using temperature as an input signal for gene expression regulation. Temperature regulation offers a robust and scalable approach for regulating gene expression in microbial engineering, thereby enhancing the production of high-value compounds with increased efficiency and yield.

3. Dynamic regulation based on endogenous signals

The dynamic regulation of gene expression can also be achieved by responding to physiological changes within the cell. This approach involves sensing changes in the internal metabolic state of cells and adjusting the metabolic pathways to maintain metabolic balance. Thus, excessive or insufficient metabolic flux, which can cause cellular stress and reduce productivity, can be avoided [45]. Physiological parameters such as cell density, intracellular metabolites, and cellular stress can be used as input signals for dynamic responses. Table 2 provides a description of recent metabolic engineering studies on *S. cerevisiae* that have employed elements responsive to endogenous signals.

3.1. Quorum-sensing system

Cell-to-cell communication is a widespread phenomenon that influences the performance of individual cells and their interactions within

Table 2
Application of endogenous signal-responsive elements in metabolic engineering of *S. cerevisiae*.

Signal type	Signal input	Response element	Product	Result	Refs.
Cell density	Cytokinin isopentenyladenine	P_{SSRE}	α-farnesene	1.8-fold	[71]
	S. cerevisiae α-pheromone	Ste12, P_{FUS1}	GFP	7.6-fold	[72]
	S. cerevisiae α-pheromone	Ste12, P_{FUS1}	para-hydroxybenzoic acid	1.1 mM	[73]
	Kluyveromyces lactis α-pheromone	Ste12, P_{FUS1}	2, -fucosyllactose 3-fucosllactose	32.05 g/L 20.91 g/L	[74]
Metabolite	Malonyl-CoA	fapO/FapR	3-hydroxypropionic acid	1 g/L	[28]
	Malonyl-CoA	fapO/FapR	GFP	95-fold	[75]
Stress	Oxidative stress	Yap1, P_{TRX2}	GFP	5.5-fold	[76]
	Oxidative stress	Yap1, P_{TRX2}	mCherry	21-fold	[77]
	Unfolded protein	P_{KAR2}, P_{PDI1}	antibody	10-fold	[78]
	Unfolded protein	P_{HAC1}, P_{KAR2}	xylanase	124.21 U/g-DCW	[79]
	Unfolded protein	P_{4xUPRE}	α-amylase	1.6-fold	[80]

an entire cell population. However, the impact of intrinsic and extrinsic environmental noises on gene expression can make it challenging to consistently coordinate the behavior of cell populations, even when they have the same genotype. By studying the communication systems between cells in nature and using synthetic biology methods to construct an artificial cell-to-cell communication system, the overall coordination performance of the system can be improved [81]. The principle behind cell-to-cell communication is that as cell density increases, the concentration of self-inducing substances secreted by the cells also increases. When the concentrations of self-inducing substances reach a certain threshold, they bind to regulatory proteins to form complexes that drive downstream gene expression. This process does not require the addition of inducing substances and achieves coupled gene expression and cell growth [82].

The quorum-sensing (QS) system found in *Arabidopsis thaliana* has been successfully adapted for use in *S. cerevisiae*. Specifically, yeast cells can be engineered to secrete cytokinin isopentenyladenine (IP) from *A. thaliana* into the extracellular environment and the secreted IP can be sensed by neighboring cells. As the density of yeast cells increased, the accumulated IP bound to the cytokinin receptor AtCRE1 and activated the endogenous Ypd1-Skn7 signaling pathway. Consequently, the synthetic *SKN7* response element (SSRE) promoter P_{SSRE} was activated [83]. To further improve this system, Yang et al. [71] introduced an auxin-inducible degron system to achieve dynamic degradation of the key squalene synthase, Erg9, which increased the production of α-farnesene by 80%. This is the first successful development of a QS-mediated dynamic protein degradation system to regulate metabolic pathways in *S. cerevisiae*. The complex regulatory mechanisms of yeast TFs may limit the activation efficiency of the *Arabidopsis* QS system in yeast, leading to a narrow dynamic range of gene regulation. Adjusting the expression strength of *SKN7* or fusing it with transcription activators may not be adequate to overcome this limitation. Therefore, further investigation is required to explore the integration of other eukaryotic QS systems in yeast.

Saccharomyces cerevisiae has a natural intercellular communication system. Haploid *S. cerevisiae* cells can be classified as either "a" or "α" type, and it recognizes different mating cells in its vicinity by sending mating-type-specific small peptide pheromones [84]. When the concentration of mating-type-specific pheromones reaches a threshold and binds to the membrane receptor Ste2, it activates the mitogen-activated protein kinase (MAPK) signaling pathway, which leads to cell cycle arrest at the G1 phase by Far1. Additionally, approximately 200 genes are induced by the transcriptional activator Ste12 binding to the 5′-(A/T)GAAACA-3′ pheromone response element upstream of the promoter [85,86].

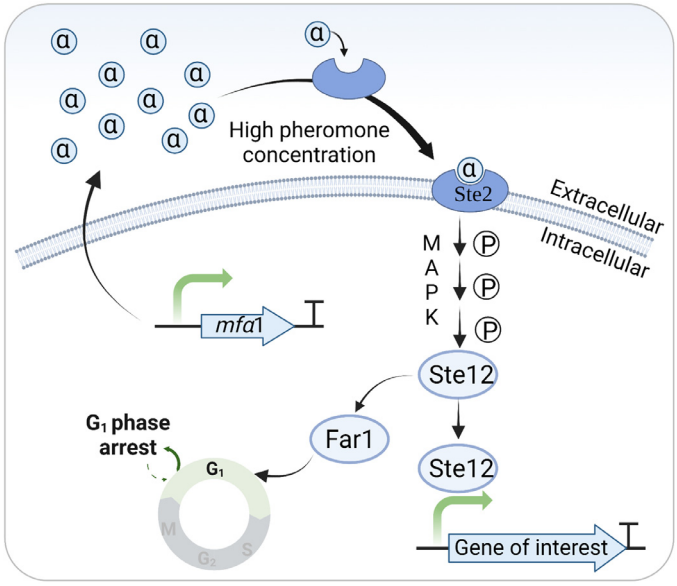

Fig. 3. Quorum-sensing (QS) dynamic control system mediated by α-pheromone [72]. The concentration of α-pheromone secreted by cells increases as cell density increases. The binding of α-pheromone to the membrane receptor Ste2 activates the MAPK signaling pathway, leading to derepression of Ste12, which subsequently activates the transcription of the pheromone-responsive promoter P_{FUS1}.

Based on this principle, Williams et al. [72] constructed a QS system in a-type haploid yeast cells (Fig. 3). A positive feedback loop was constructed by controlling the expression of α-pheromones and GFP through the responsive promoter P_{FUS1}. The concentration of α-pheromones increases as cell density increases, leading to increased expression of GFP and α-pheromones. When α-pheromone was expressed under the response of the aromatic amino acid promoter P_{ARO9}, the pheromone response pathway could be adjusted by modulating the type and concentration of aromatic amino acids. This approach provides increased flexibility in modulating gene expression in response to changes in cell density and environmental factors, which can be advantageous for optimizing bioproduction processes. However, this system lacks mechanisms to suppress or silence gene expression. To address this limitation, a QS system was combined with RNA interference (RNAi) to biosynthesize para-hydroxybenzoic acid (PHBA). Introducing Dicer and Argonaute proteins facilitated mRNA degradation, thereby enabling the silencing of genes

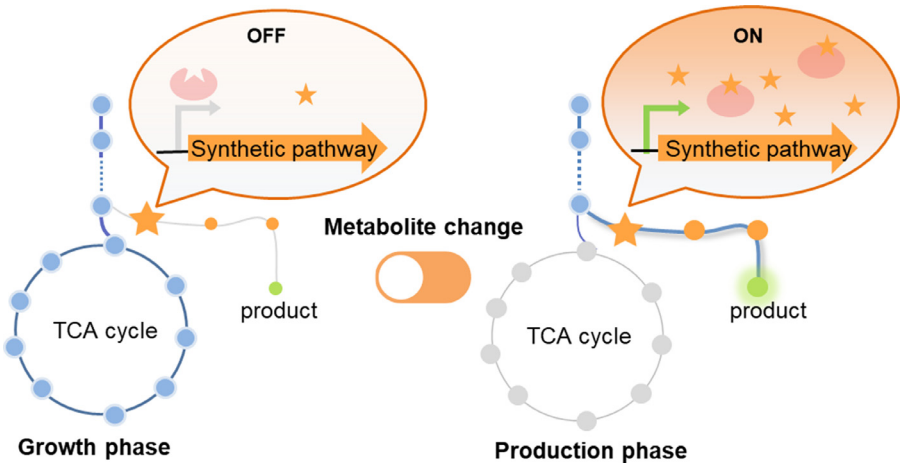

Fig. 4. Dynamic control system via metabolite response. Biosensors are used to control key nodes in metabolic pathways by responding to metabolites changes. This enables the dynamic control of metabolic fluxes between cell growth phases and production phases.

involved in competing pathways. This approach resulted in increased production of PHBA, yielding 1.1 mM of PHBA [73].

The membrane receptor Ste2 plays a central role in the signal transduction network of *S. cerevisiae*. It exhibits varying affinities for different pheromones, enabling the precise regulation of the pheromone response pathway. Di Roberto et al. [87] found that the pheromone receptor Ste2p displayed higher sensitivity to α-pheromone from *S. cerevisiae* (Sα) compared to the α-pheromone from *Kluyveromyces lactis* (Kα). To minimize the conflict between the growth and production phases, Xu et al. [74] constructed an auto inducible gene expression system based on the yeast pheromone response pathway. They used Kα, which has a lower affinity than that of the native Sα pheromone, to mitigate growth reduction caused by the early onset of the pheromone response. Furthermore, the system was enhanced by deleting *GAL80* and targeting pathway genes using the transcriptional activator Gal4p, which was expressed under the control of the pheromone-responsive promoter P_{FUS1}. When this system was applied to the production of human milk oligosaccharides, the yields of 2′-fucosyllactose and 3-fucosllactose increased by 147% and 153%, respectively, compared to using the *GAL* promoter alone. Genetic programs represent a promising approach for implementing dynamic regulation in yeast and can be used to produce biochemicals that may impose a heavy metabolic burden on cell growth.

3.2. Metabolite-responsive dynamic regulation

When yeast is used to synthesize chemicals or natural products, a coordination in the expression of multiple genes in metabolic pathways is often necessary to increase the yield. This process involves the selection, modification, and assembly of a large number of different elements [88,89]. Metabolite biosensors are increasingly utilized to dynamically regulate the expression of key genes or modules in metabolic pathways. These biosensors facilitate the real-time monitoring of intracellular metabolite levels, enabling precise adjustment of gene expression according to the actual metabolic state of the strain. This capability allows enhanced control over cell growth and metabolic flux, leading to improved optimization of the production process [90]. Key nodes in metabolic pathways are identified and regulated using biological sensors that respond to changes in the concentrations of key metabolites. Optimizing the regulation of these key metabolic nodes allows for improved efficiency of both upstream precursor synthesis and downstream product conversion, resulting in higher yields of the desired product while minimizing the metabolic burden on the cell (Fig. 4). Acetyl-CoA and malonyl-CoA, which are important central metabolites in yeast, also serve as precursors in the production of various chemicals [91]. For example, dynamic regulation of 3HP synthesis was achieved through a two-stage approach using the high-concentration glucose-inducible promoter P_{HXT1} combined with the *fapO*/FapR system. This system specifically detects changes in malonyl-CoA concentration to effectively control carbon metabolism [46]. As the fermentation process progressed and the glucose concentration gradually decreased, the low concentration of glucose weakened the expression intensity of P_{HXT1}, resulting in reduced fat synthesis pathway activity. The continuously accumulating acetyl-CoA relieved transcriptional repression by binding to FapR, leading to the dynamic activation of acetyl-CoA reductase MCRCα. This resulted in a nearly 10-fold increase in the production of 3HP, reaching 1 g/L. Recently, Qiu et al. [92] observed that overexpression of *UGP1* has a buffering effect that dynamically regulates carbon flux. Specifically, in the presence of excess glucose, carbon is directed towards glycogen for storage. Conversely, stored carbohydrates are consumed during periods of low glucose levels, thereby providing more carbon for malonyl-CoA synthesis.

Although many natural biosensors exist, their inherent limitations in terms of basal expression strength and narrow response range to metabolites restrict their applications in metabolic engineering. To utilize these biosensors effectively, researchers often need to modify them to meet the specific requirements of the application environment. This may involve optimizing factors such as the basal expression strength, induction output strength, induction dynamic range, and response sensitivity of the biosensor [93,94]. Common modifications to these biosensors include replacing core promoter elements with different basal expression strengths, adjusting the copy number of response elements, and changing the types and spatial positions of response elements in the promoter [94–97]. For example, in the *fapO*/FapR system, placing the TFBS in the core promoter region can increase the dynamic regulation range of the promoter but can also cause a spatial hindrance effect that results in a decrease in the basal expression level of the promoter. In contrast, moving the TFBS upstream of the promoter and fusing the yeast transcriptional repressor Mig1 with FapR effectively increases the dynamic repression strength of the promoter without significantly affecting the basal expression level [75].

3.3. Stress-responsive dynamic regulation

Cells possess stress-response systems that enable them to adapt to environmental changes. During the stress response, external stress signals are detected by the cell and converted into intracellular signals, which are then transmitted through signal transduction pathways to activate TFs that regulate the expression of target genes [98,99]. The regulation of gene expression in response to stress signals allows for the fine-tuning of gene expression levels in response to the intensity of the stress signal, which helps avoid problems such as metabolic imbalances resulting from continuous high-intensity expression or insufficient expression. By identifying and characterizing the stress response promoters and their

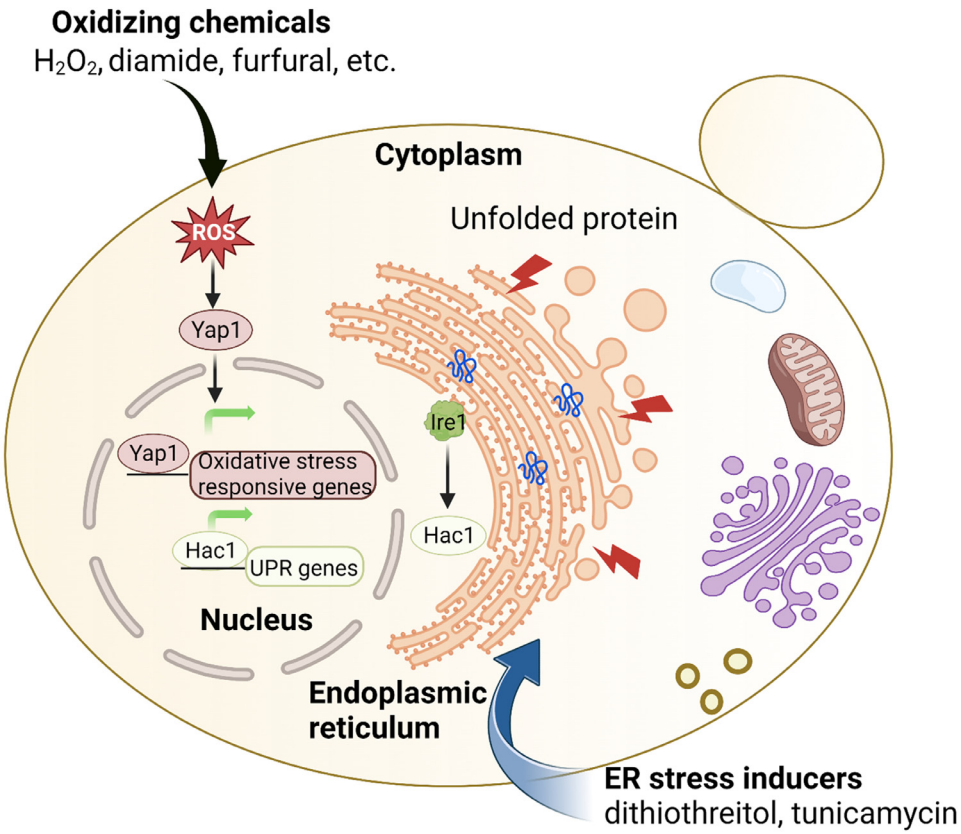

Fig. 5. Dynamic regulation of metabolism by oxidation response elements. In response to oxidizing inducers or unfolded proteins, stress signals are transmitted from the cytoplasm or endoplasmic reticulum to nucleus, triggering gene expression.

response sensitivities, these promoters can be harnessed to dynamically regulate gene expression and improve the yield of target products.

There are three main pathways through which reactive oxygen species (ROS) are generated during aerobic cellular metabolism: the oxidative phosphorylation of the respiratory chain in mitochondria, β-oxidation of fatty acids in peroxisomes, and oxidation of proteins in the endoplasmic reticulum (ER) [100]. When cells are unable to cope with the damage caused by ROS using conventional antioxidant systems, an oxidative stress response is triggered. The oxidative stress response is mainly regulated by the transcriptional activation factors Yap1 and Skn7 [98]. Biosensors that utilize cellular responses to oxidative stress have been developed. These biosensors are designed to detect changes in oxidative stress levels within cells. They enable the detection of target compounds, monitoring of cell states, and the regulation of gene expression to optimize metabolic processes (Fig. 5). Zhang et al. [76] constructed a redox biosensor that could detect changes in the NADPH/$NADP^+$ ratio in real time by modifying the transcription-binding site of Yap1 upstream of the thioredoxin promoter P_{TRX2}. This biosensor was used to screen cells with high NADPH/$NADP^+$ ratios from a yeast library for potential biosynthetic applications. However, a potential limitation of this biosensor is the length of the engineered promoter, which is approximately 700 base pairs (bp). This length may affect efficiency and restrict its applicability to certain systems. Dacquay et al. [77] introduced tandem Yap1 binding sites, particularly 5′-TTACTAA-3′, in the upstream of the minimal core promoter. By optimizing the number and spacing of binding sequences, the length of 5′-UTR, and the inclusion of two alternative binding sites, they successfully engineered a Yap1-dependent oxidative stress-type promoter of only 171 bp in length. The compact promoter exhibits heightened responsiveness to oxidative stress. This study provides valuable guidance for the design and optimization of TF-based biosensors.

Heterologous protein expression often induces cellular stress [101]. When excessive amounts of misfolded proteins accumulate in the ER, they trigger ER stress and activate the unfolded protein response (UPR) [102]. The UPR is a cellular stress response pathway aimed at restoring ER homeostasis by enhancing ER protein processing and folding capacity as well as reducing the burden of misfolded proteins within the ER. Once the UPR is activated, the stress signal is transmitted through the transmembrane-sensing protein Ire1, which is located in the ER membrane. Activated Ire1 splices *HAC1* precursor mRNA to generate mature mRNA. Mature *HAC1* mRNA is translated into the Hac1 TF, which translocates to the nucleus and interacts with the unfolded protein response element (UPRE), a cis-acting regulatory element present in the promoters of UPR target genes. This interaction leads to transcriptional changes in more than 400 genes including *KAR2, PDI1*, and *ERO1* [78]. By utilizing the promoters of these genes to regulate the expression of molecular chaperone elements, the expression intensity of the molecular chaperones can be dynamically adjusted to match the level of ER stress and increase heterologous protein production [103]. Moreover, the promoter-mediated expression of fluorescent proteins can be used to construct UPR biosensors that can detect ER stress caused by the accumulation of unfolded proteins. These biosensors can be used to effectively screen cellulases at low secretion pressures. Compared to the traditional detection methods based on *HAC1* mRNA splicing, this type of biosensor has a higher sensitivity and a wider response range [79]. Benisch et al. [80] developed and characterized yeast strains for optogenetic regulation of protein production, along with concurrent monitoring of the UPR. In their study, the light-responsive protein Msn2AD-EL222 monomer was utilized, as it undergoes reversible dimerization under blue light. The dimerized form can bind to the Msn2AD-EL222 binding site and activate the transcription of the P_{EL222} promoter. This promoter drives the expression of α-amylase and a transcriptional reporter that dynamically measures α-amylase transcription. A UPR sensor was also constructed to simultaneously monitor protein expression pressure. Using a fully automated customized photobioreactor and UPR-based real-time feedback measurement, researchers have achieved op-

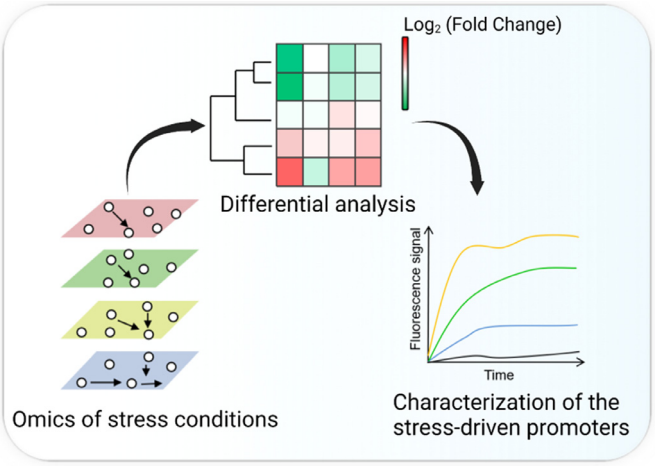

Fig. 6. Discovery of stress-responsive promoters by omics-based tools.

togenetic modulation of α-amylase expression in yeast. This allowed for controlling the UPR level and attaining a desired set point, resulting in a 60% increase in product titers. This approach combines the characterization of the production process with closed-loop real-time feedback, leading to reduced overall turnaround and upscaling times.

4. Omics approaches to discover response elements

Natural biosensors exhibit specificity towards metabolites, and there is a need for more biosensors for dynamic control applications. Therefore, a general approach is required to discover new metabolite sensors. One possible approach is to exploit endogenous response systems within cells, in which the accumulation of metabolites beyond a certain threshold triggers a stress response (Fig. 6). For instance, responsive promoters can be identified using omics techniques by inducing cells to activate stress response pathways through metabolite accumulation or exposure to stressful conditions. This approach offers the advantage of utilizing endogenous TFs for dynamically regulating target genes, thereby eliminating the need to design additional TFs. As a result, it enables the precise control of metabolic pathways.

Researchers discovered an FPP-responsive promoter using high-throughput genomics. This promoter has been used to dynamically regulate the synthesis and conversion of intermediates to match the cellular state, resulting in increased amorphadiene production in *E. coli* [104]. Medium-chain fatty acids (MCFAs), which consist of chains of 6–12 carbon atoms (C6–C12), are valuable chemicals used in lubricants, detergents, and cosmetics and offer advantages over long-chain fatty acids (LCFAs), such as improved biofuel quality and potential as fossil fuel substitutes [105]. To identify the endogenous promoters responsive to MCFAs, Han et al. [106] conducted a transcriptomic analysis of yeast strains cultivated in a culture medium supplemented with fatty acids (C6, C12, and C16). The transcriptome data were categorized into upregulated and downregulated genes. Among the upregulated genes, those particularly responding to C6 or C12 fatty acids, but not to C16 fatty acids, were selected as potential MCFA-responsive promoters. A similar screening strategy was employed for downregulated genes, and seven MCFA-responsive promoters were identified. Some of these promoters exhibit high sensitivity, responding to concentrations as low as 0.02 mM of fatty acids [106]. These fatty acid-responsive promoters can be used for dynamically regulating fatty acid and fatty acid derivative synthesis. Similarly, Gao et al. [107] performed a comparative transcriptome analysis of wild-type fatty acid-producing strains and strains engineered to accumulate acyl-CoA and identified endogenous fatty acid/acyl-CoA-responsive promoters. The differentially expressed genes served as the basis for identifying potential fatty acid/acyl-CoA-responsive promoters. The dynamic regulation of *MaFAR1* using one of the identified fatty acid/acyl-CoA-responsive promoters, along with enhanced precursor and cofactor supply and the promotion of peroxisome biogenesis, enables the coordinated spatiotemporal regulation of fatty alcohol biosynthesis.

In addition to accumulating intracellular metabolites, microorganisms also experience stress under adverse conditions. Enhancing cell adaptability and tolerance is crucial for industrial applications. During ethanol production from lignocellulosic feedstocks, yeast cells are subjected to multiple stresses, including high temperatures and toxic substances, in the lignocellulosic pretreatment liquor. These stresses can result in the generation of a large amount of ROS and disruption of cellular metabolic homeostasis [108,109]. Genes are often overexpressed using strong constitutive promoters to enhance tolerance and adaptability to such stress. However, this approach may overlook potential interactions between the environment and strain. Qin et al. [110] conducted a study in which *S. cerevisiae* was cultivated under non-stress (30 ˚C, 2% glucose) and stress conditions (36 ˚C, 10% glucose). Transcriptomes were compared between the two conditions to identify differentially expressed genes. Genes with higher transcript levels under stress conditions were classified as stress-driven, whereas those with the opposite pattern were classified as stress-repressed. Stress-driven promoters promote glutathione (GSH) synthesis, whereas stress-repressed promoters facilitate acetate degradation and improve cell tolerance to ROS and acetic acid. In an industrial lignocellulosic medium, the engineered strain showed a 17.5% increase in ethanol production at 36 ˚C compared to the control strain [110]. Using stress-responsive promoters enables the dynamic feedback regulation of genes, resulting in strong gene expression under stress and weak gene expression under non-stress conditions. This establishes a relationship between the metabolic state of the cell and heterologous metabolic pathways. The identification of response elements using omics approaches offers a promising solution for dynamically regulating microbial cell factories.

5. Summary

This review highlights the recent advancements in the dynamic regulation of metabolic pathways in *S. cerevisiae* to enhance the production of valuable products. Dynamic regulation involves modification of the binding affinity of TFs to their respective TFBSs in response to signals. TF-based biosensors offer versatility in detecting various signals such as chemical inducers, light, temperature, cell density, metabolites, and stress. Light and temperature have the advantages of controllability and reversibility, making them suitable for the dynamic regulation and periodic reciprocating control of physiological metabolism.

However, there are several challenges that necessitate further research. There is a need to enhance the performance and diversity of the sensing elements. Optimal determination of induction intensity and time is essential for exogenous signal regulation systems, considering the potential lag effect between signal changes and signal output. In the future, a combination of rapid cell-state detection using biosensors and computer-aided closed-loop feedback will facilitate the development of more robust and responsive dynamic regulation tools. This advancement will unlock new opportunities for the efficient production of chemicals and proteins.

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.

CRediT authorship contribution statement

Chufan Xiao: Conceptualization, Writing – original draft, Writing – review & editing. **Yuyang Pan:** Conceptualization, Writing – review &

editing. **Mingtao Huang:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

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References

[1] S.K. Nandy, R.K. Srivastava, A review on sustainable yeast biotechnological processes and applications, Microbiol. Res. 207 (2018) 83–90.
[2] J. Nielsen, Production of biopharmaceutical proteins by yeast: advances through metabolic engineering, Bioengineered 4 (4) (2013) 207–211.
[3] I. Borodina, J. Nielsen, Advances in metabolic engineering of yeast *Saccharomyces cerevisiae* for production of chemicals, Biotechnol. J. 9 (5) (2014) 609–620.
[4] S. Kwak, et al., Redirection of the glycolytic flux enhances isoprenoid production in *Saccharomyces cerevisiae*, Biotechnol. J. 15 (2) (2020) e1900173.
[5] Y. Zhao, et al., Production of *β*-carotene in *Saccharomyces cerevisiae* through altering yeast lipid metabolism, Biotechnol. Bioeng. 118 (5) (2021) 2043–2052.
[6] T. Tong, et al., A biosynthesis pathway for 3-hydroxypropionic acid production in genetically engineered *Saccharomyces cerevisiae*, Green Chem. (2021).
[7] J. Lian, S. Mishra, H. Zhao, Recent advances in metabolic engineering of *Saccharomyces cerevisiae*: new tools and their applications, Metab. Eng. 50 (2018) 85–108.
[8] D. Liu, et al., Dynamic metabolic control: towards precision engineering of metabolism, J. Ind. Microbiol. Biotechnol. 45 (7) (2018) 535–543.
[9] X. Shen, et al., Dynamic gene expression engineering as a tool in pathway engineering, Curr. Opin. Biotechnol. 59 (2019) 122–129.
[10] X.Y. Ge, et al., Regulation of metabolic flux in Lactobacillus casei for lactic acid production by overexpressed ldhL gene with two-stage oxygen supply strategy, J. Microbiol. Biotechnol. 25 (1) (2015) 81–88.
[11] Y. Teng, et al., Biosensor-enabled pathway optimization in metabolic engineering, Curr. Opin. Biotechnol. 75 (2022) 102696.
[12] F. Guo, et al., Metabolic engineering of Pichia pastoris for malic acid production from methanol, Biotechnol. Bioeng. 118 (1) (2021) 357–371.
[13] Y. Zhu, et al., Development of bifunctional biosensors for sensing and dynamic control of glycolysis flux in metabolic engineering, Metab. Eng. 68 (2021) 142–151.
[14] X. Chen, L. Liu, Gene circuits for dynamically regulating metabolism, Trends Biotechnol. 36 (8) (2018) 751–754.
[15] Y. Yang, et al., Sensor-regulator and RNAi based bifunctional dynamic control network for engineered microbial synthesis, Nat. Commun. 9 (1) (2018) 3043.
[16] H.T. Zhai, et al., CRISPR-mediated protein-tagging signal amplification systems for efficient transcriptional activation and repression in *Saccharomyces cerevisiae*, Nucleic Acids Res. 50 (10) (2022) 5988–6000.
[17] Z.Y. Lu, et al., Auxin-mediated protein depletion for metabolic engineering in terpene-producing yeast, Nat. Commun. 12 (1) (2021) 1051.
[18] Y. Chen, et al., Advances in RNAi-assisted strain engineering in *Saccharomyces cerevisiae*, Front. Bioeng. Biotechnol. 8 (2020) 731.
[19] J. Ye, et al., Dynamic control:an efficient strategy for metabolically engineering microbial cell factories, Biotechnol. Bull. 36 (6) (2020) 1–12.
[20] Z. Yu, et al., Application of dynamic regulation strategies in metabolic engineering, Synth. Biol. J. 1 (4) (2020) 440–453.
[21] H. Zhai, et al., CRISPR-mediated protein-tagging signal amplification systems for efficient transcriptional activation and repression in *Saccharomyces cerevisiae*, Nucleic Acids Res. 50 (10) (2022) 5988–6000.
[22] J. Zhang, et al., Modular tuning engineering and versatile applications of genetically encoded biosensors, Crit. Rev. Biotechnol. 42 (7) (2022) 1010–1027.
[23] Y. Zhang, S. Shi, Transcription factor-based biosensor for dynamic control in yeast for natural product synthesis, Front. Bioeng. Biotechnol. 9 (2021) 635265.
[24] R. Wang, et al., Design and characterization of biosensors for the screening of modular assembled naringenin biosynthetic library in *Saccharomyces cerevisiae*, ACS Synth. Biol. 8 (9) (2019) 2121–2130.
[25] R. Mahr, J. Frunzke, Transcription factor-based biosensors in biotechnology: current state and future prospects, Appl. Microbiol. Biotechnol. 100 (1) (2016) 79–90.
[26] J. Tellechea-Luzardo, M.T. Stiebritz, P. Carbonell, Transcription factor-based biosensors for screening and dynamic regulation, Front. Bioeng. Biotechnol. 11 (2023) 1118702.
[27] B. De Paepe, et al., Tailor-made transcriptional biosensors for optimizing microbial cell factories, J. Ind. Microbiol. Biotechnol. 44 (4–5) (2017) 623–645.
[28] J. Maury, et al., Glucose-dependent promoters for dynamic regulation of metabolic pathways, Front. Bioeng. Biotechnol. 6 (2018) 63.
[29] Q. Bian, et al., Hierarchical dynamic regulation of *Saccharomyces cerevisiae* for enhanced lutein biosynthesis, Biotechnol. Bioeng. 120 (2) (2023) 536–552.
[30] H. Chen, et al., High production of valencene in *Saccharomyces cerevisiae* through metabolic engineering, Microb. Cell Fact 18 (1) (2019) 195.
[31] M. Zhao, et al., Pathway engineering in yeast for synthesizing the complex polyketide bikaverin, Nat. Commun. 11 (1) (2020) 6197.
[32] X. Lv, et al., Engineered *Saccharomyces cerevisiae* for the De novo biosynthesis of (−)-menthol, J. Fungi 8 (9) (2022) 982.
[33] P.G. Teixeira, et al., Dynamic regulation of fatty acid pools for improved production of fatty alcohols in *Saccharomyces cerevisiae*, Microb. Cell Fact 16 (1) (2017) 45.
[34] P. Zhou, et al., Development of a highly efficient copper-inducible GAL regulation system (CuIGR) in *Saccharomyces cerevisiae*, ACS Synth. Biol. 10 (12) (2021) 3435–3444.
[35] A.S. Hossain, R. Teparić, V. Mrša, Comparison of two models of surface display of xylose reductase in the *Saccharomyces cerevisiae* cell wall, Enzyme Microb. Technol. 123 (2019) 8–14.
[36] P. Promdonkoy, et al., Metabolic engineering of *Saccharomyces cerevisiae* for production of fragrant terpenoids from agarwood and sandalwood, Fermentation 8 (9) (2022) 429.
[37] X. Xu, et al., A single-component optogenetic system allows stringent switch of gene expression in yeast cells, ACS Synth. Biol. 7 (9) (2018) 2045–2053.
[38] E.M. Zhao, et al., Optogenetic regulation of engineered cellular metabolism for microbial chemical production, Nature 555 (7698) (2018) 683–687.
[39] E.M. Zhao, et al., Optogenetic amplification circuits for light-induced metabolic control, ACS Synth. Biol. 10 (5) (2021) 1143–1154.
[40] M.A. Lalwani, et al., The neurospora crassa inducible Q system enables simultaneous optogenetic amplification and inversion in *Saccharomyces cerevisiae* for bidirectional control of gene expression, ACS Synth. Biol. 10 (8) (2021) 2060–2075.
[41] P. Zhou, et al., Development of a temperature-responsive yeast cell factory using engineered Gal4 as a protein switch, Biotechnol. Bioeng. 115 (5) (2018) 1321–1330.
[42] P. Zhou, et al., Directed coevolution of beta-carotene ketolase and hydroxylase and its application in temperature-regulated biosynthesis of astaxanthin, J. Agric. Food Chem. 67 (4) (2019) 1072–1080.
[43] B. Shen, et al., Fermentative production of Vitamin E tocotrienols in *Saccharomyces cerevisiae* under cold-shock-triggered temperature control, Nat. Commun. 11 (1) (2020) 5155.
[44] T. Liu, et al., Construction of a stable and temperature-responsive yeast cell factory for crocetin biosynthesis using CRISPR-Cas9, Front. Bioeng. Biotechnol. 8 (2020) 653.
[45] C.J. Hartline, et al., Dynamic control in metabolic engineering: theories, tools, and applications, Metab. Eng. 63 (2021) 126–140.
[46] F. David, J. Nielsen, V. Siewers, Flux control at the malonyl-CoA node through hierarchical dynamic pathway regulation in *Saccharomyces cerevisiae*, ACS Synth. Biol. 5 (3) (2016) 224–233.
[47] J. Horak, Regulations of sugar transporters: insights from yeast, Curr. Genet. 59 (2013) 1–31.
[48] W. Xie, et al., Construction of a controllable beta-carotene biosynthetic pathway by decentralized assembly strategy in *Saccharomyces cerevisiae*, Biotechnol. Bioeng. 111 (1) (2014) 125–133.
[49] P.J. Westfall, et al., Production of amorphadiene in yeast, and its conversion to dihydroartemisinic acid, precursor to the antimalarial agent artemisinin, Proc. Natl. Acad. Sci. U. S. A. 109 (3) (2012) 111–118.
[50] Y. Liu, et al., De novo production of hydroxytyrosol by metabolic engineering of *Saccharomyces cerevisiae*, J. Agric. Food Chem. 70 (24) (2022) 7490–7499.
[51] J. Zou, et al., Uncoupling glucose sensing from GAL metabolism for heterologous lactose fermentation in *Saccharomyces cerevisiae*, Biotechnol. Lett. 43 (8) (2021) 1607–1616.
[52] N.A Da Silva, S. Srikrishnan, Introduction and expression of genes for metabolic engineering applications in *Saccharomyces cerevisiae*, FEMS Yeast Res. 12 (2) (2012) 197–214.
[53] S. Shibasaki, M. Ueda, Progress of molecular display technology using *Saccharomyces cerevisiae* to achieve sustainable development goals, Microorganisms 11 (1) (2023) 125.
[54] M. Sabzevari, et al., Strain design optimization using reinforcement learning, PLoS Comput. Biol. 18 (6) (2022) e1010177.
[55] L. Qin, et al., Mining and design of biosensors for engineering microbial cell factory, Curr. Opin. Biotechnol. 75 (2022) 102694.
[56] M.A. Lalwani, E.M. Zhao, J.L. Avalos, Current and future modalities of dynamic control in metabolic engineering, Curr. Opin. Biotechnol. 52 (2018) 56–65.
[57] T. Drepper, et al., Lights on and action! Controlling microbial gene expression by light, Appl. Microbiol. Biotechnol. 90 (1) (2011) 23–40.
[58] X. Wang, et al., Reversible thermal regulation for bifunctional dynamic control of gene expression in Escherichia coli, Nat. Commun. 12 (1) (2021) 1411.
[59] C. Carrasco-López, et al., Optogenetics and biosensors set the stage for metabolic cybergenetics, Curr. Opin. Biotechnol. 65 (2020) 296–309.
[60] J.M. An-Adirekkun, et al., A yeast optogenetic toolkit (yOTK) for gene expression control in *Saccharomyces cerevisiae*, Biotechnol. Bioeng. 117 (3) (2020) 886–893.
[61] X. Wang, X. Chen, Y. Yang, Spatiotemporal control of gene expression by a light-switchable transgene system, Nat. Methods 9 (3) (2012) 266–269.
[62] J.E. Toettcher, et al., The promise of optogenetics in cell biology: interrogating molecular circuits in space and time, Nat. Methods 8 (1) (2011) 35–38.
[63] J.G. Mena, S. Kumar, M. Khammash, Dynamic cybergenetic control of bacterial co–culture composition via optogenetic feedback, Nat. Commun. 13 (1) (2022) 4808.
[64] K. Deisseroth, Optogenetics: 10 years of microbial opsins in neuroscience, Nat. Neurosci. 18 (9) (2015) 1213–1225.
[65] R.A. Kramer, et al., Regulated expression of a human interferon gene in yeast: control by phosphate concentration or temperature, Proc. Natl. Acad. Sci. U. S. A. 81 (2) (1984) 367–370.
[66] A.Z. Sledziewski, et al., Superimposition of temperature regulation on yeast promoters, Methods Enzymol. 185 (1990) 351–366.
[67] G. Chakshusmathi, et al., Design of temperature-sensitive mutants solely from amino acid sequence, Proc. Natl. Acad. Sci. U. S. A. 101 (21) (2004) 7925–7930.
[68] M.N. Stagoj, A. Comino, R. Komel, Fluorescence based assay of GAL system in yeast *Saccharomyces cerevisiae*, FEMS Microbiol. Lett. 244 (1) (2005) 105–110.

[69] E. Martegani, et al., Expression of high levels of human tissue plasminogen activator in yeast under the control of an inducible GAL promoter, Appl. Microbiol. Biotechnol. 37 (5) (1992) 604–608.
[70] W. Xie, et al., Sequential control of biosynthetic pathways for balanced utilization of metabolic intermediates in *Saccharomyces cerevisiae*, Metab. Eng. 28 (2015) 8–18.
[71] X. Yang, et al., Quorum sensing-mediated protein degradation for dynamic metabolic pathway control in *Saccharomyces cerevisiae*, Metab. Eng. 64 (2021) 85–94.
[72] T.C. Williams, L.K. Nielsen, C.E. Vickers, Engineered quorum sensing using pheromone-mediated cell-to-cell communication in *Saccharomyces cerevisiae*, ACS Synth. Biol. 2 (3) (2013) 136–149.
[73] T.C. Williams, et al., Quorum-sensing linked RNA interference for dynamic metabolic pathway control in *Saccharomyces cerevisiae*, Metab. Eng. 29 (2015) 124–134.
[74] M. Xu, et al., Engineering pheromone-mediated quorum sensing with enhanced response output increases fucosyllactose production in *Saccharomyces cerevisiae*, ACS Synth. Biol. 12 (1) (2023) 238–248.
[75] Y. Dabirian, et al., Expanding the dynamic range of a transcription factor-based biosensor in *Saccharomyces cerevisiae*, ACS Synth. Biol. 8 (9) (2019) 1968–1975.
[76] J. Zhang, et al., Engineering an NADPH/NADP(+) redox biosensor in yeast, ACS Synth. Biol. 5 (12) (2016) 1546–1556.
[77] L.C. Dacquay, D.R. McMillen, Improving the design of an oxidative stress sensing biosensor in yeast, FEMS Yeast Res. 21 (4) (2021) foab025.
[78] D. Sarkar, S. Paira, B. Das, Nuclear mRNA degradation tunes the gain of the unfolded protein response in *Saccharomyces cerevisiae*, Nucleic Acids Res. 46 (3) (2018) 1139–1156.
[79] G. Cedras, et al., The *in vivo* detection and measurement of the unfolded protein response in recombinant cellulase producing *Saccharomyces cerevisiae* strains, Biotechnol. Appl. Biochem. 67 (1) (2020) 82–94.
[80] M. Benisch, et al., Optogenetic closed-loop feedback control of the unfolded protein response optimizes protein production, Metab. Eng. 77 (2023) 32–40.
[81] C. Ge, et al., Redesigning regulatory components of quorum-sensing system for diverse metabolic control, Nat. Commun. 13 (1) (2022) 4808.
[82] E.M. Kim, et al., Autonomous control of metabolic state by a quorum sensing (QS)-mediated regulator for bisabolene production in engineered E. coli, Metab. Eng. 44 (2017) 325–336.
[83] M.T. Chen, R. Weiss, Artificial cell-cell communication in yeast *Saccharomyces cerevisiae* using signaling elements from Arabidopsis thaliana, Nat. Biotechnol. 23 (12) (2005) 1551–1555.
[84] S. Hennig, G. Rodel, K. Ostermann, Artificial cell-cell communication as an emerging tool in synthetic biology applications, J. Biol. Eng. 9 (2015) 13.
[85] L. Bardwell, A walk-through of the yeast mating pheromone response pathway, Peptides 26 (2) (2005) 339–350.
[86] R. Harrison, C. DeLisi, Condition specific transcription factor binding site characterization in *Saccharomyces cerevisiae*, Bioinformatics 18 (10) (2002) 1289–1296.
[87] R.B. Di Roberto, et al., Evolution of a G protein-coupled receptor response by mutations in regulatory network interactions, Nat. Commun. 7 (1) (2016) 12344.
[88] A. Krivoruchko, J. Nielsen, Production of natural products through metabolic engineering of *Saccharomyces cerevisiae*, Curr. Opin. Biotechnol. 35 (2015) 7–15.
[89] L.B. Pickens, Y. Tang, Y.H. Chooi, Metabolic engineering for the production of natural products, Annu. Rev. Chem. Biomol. Eng. 2 (2011) 211–236.
[90] C. Qiu, H. Zhai, J. Hou, Biosensors design in yeast and applications in metabolic engineering, FEMS Yeast Res. 19 (8) (2019) foz082.
[91] S. Li, et al., Recent progress in metabolic engineering of *Saccharomyces cerevisiae* for the production of malonyl-CoA derivatives, J. Biotechnol. 325 (2021) 83–90.
[92] C. Qiu, et al., Dynamic-tuning yeast storage carbohydrate improves the production of acetyl-CoA-derived chemicals, iScience 26 (1) (2023) 105817.
[93] J.K. Rogers, N.D. Taylor, G.M. Church, Biosensor-based engineering of biosynthetic pathways, Curr. Opin. Biotechnol. 42 (2016) 84–91.
[94] A.S. Rajkumar, et al., Engineering of synthetic, stress-responsive yeast promoters, Nucleic Acids Res. 44 (17) (2016) e136.
[95] H. Tang, et al., Promoter architecture and promoter engineering in *Saccharomyces cerevisiae*, Metabolites 10 (8) (2020) 320.
[96] H. Redden, H.S. Alper, The development and characterization of synthetic minimal yeast promoters, Nat. Commun. 6 (2015) 7810.
[97] C. Qiu, et al., Engineering transcription factor-based biosensors for repressive regulation through transcriptional deactivation design in *Saccharomyces cerevisiae*, Microb. Cell Fact 19 (1) (2020) 146.
[98] A. Ikner, K. Shiozaki, Yeast signaling pathways in the oxidative stress response, Mutat. Res. 569 (1–2) (2005) 13–27.
[99] Y. Wu, et al., Yeast cell fate control by temporal redundancy modulation of transcription factor paralogs, Nat. Commun. 12 (1) (2021) 3145.
[100] E. Herrero, et al., Redox control and oxidative stress in yeast cells, Biochim. Biophys. Acta 1780 (11) (2008) 1217–1235.
[101] Y. Lin, et al., Improved protein production in yeast using cell engineering with genes related to a key factor in the unfolded protein response, Metab. Eng. 77 (2023) 152–161.
[102] C.L. Young, A.S. Robinson, Protein folding and secretion: mechanistic insights advancing recombinant protein production in S. cerevisiae, Curr. Opin. Biotechnol. 30 (2014) 168–177.
[103] J.C. de Ruijter, E.V. Koskela, A.D. Frey, Enhancing antibody folding and secretion by tailoring the *Saccharomyces cerevisiae* endoplasmic reticulum, Microb. Cell Fact 15 (2016) 87.
[104] R.H. Dahl, et al., Engineering dynamic pathway regulation using stress-response promoters, Nat. Biotechnol. 31 (11) (2013) 1039–1046.
[105] P. Xu, et al., Engineering Yarrowia lipolytica as a platform for synthesis of drop-in transportation fuels and oleochemicals, Proc. Natl. Acad. Sci. U. S. A. 113 (39) (2016) 10848–10853.
[106] L. Han, et al., Discovery and identification of medium-chain fatty acid responsive promoters in *Saccharomyces cerevisiae*, Eng. Life Sci 20 (5–6) (2020) 186–196.
[107] N. Gao, et al., Spatial-temporal regulation of fatty alcohol biosynthesis in yeast, Biotechnol. Biofuels Bioprod. 15 (1) (2022) 141.
[108] K. Xu, et al., Multilevel defense system (MDS) relieves multiple stresses for economically boosting ethanol production of industrial *Saccharomyces cerevisiae*, ACS Energy Lett. 5 (2) (2020) 572–582.
[109] C. Auesukaree, Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation, J. Biosci. Bioeng. 124 (2) (2017) 133–142.
[110] L. Qin, et al., Stress-driven dynamic regulation of multiple tolerance genes improves robustness and productive capacity of *Saccharomyces cerevisiae* in industrial lignocellulose fermentation, Metab. Eng. 61 (2020) 160–170.