Critical Reviews in Biotechnology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ibty20

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To cite this article: Shenghu Zhou, Hal S. Alper, Jingwen Zhou & Yu Deng (2022): Intracellular biosensor-based dynamic regulation to manipulate gene expression at the spatiotemporal level, Critical Reviews in Biotechnology, DOI: 10.1080/07388551.2022.2040415

To link to this article: https://doi.org/10.1080/07388551.2022.2040415

Published online: 21 Apr 2022.

CRITICAL REVIEWS IN BIOTECHNOLOGY
https://doi.org/10.1080/07388551.2022.2040415

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REVIEW ARTICLE

Intracellular biosensor-based dynamic regulation to manipulate gene expression at the spatiotemporal level

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ABSTRACT

The use of intracellular, biosensor-based dynamic regulation strategies to regulate and improve the production of useful compounds have progressed significantly over previous decades. By employing such an approach, it is possible to simultaneously realize high productivity and optimum growth states. However, industrial fermentation conditions contain a mixture of high- and low-performance non-genetic variants, as well as young and aged cells at all growth phases. Such significant individual variations would hinder the precise controlling of metabolic flux at the single-cell level to achieve high productivity at the macroscopic population level. Intracellular biosensors, as the regulatory centers of metabolic networks, can real-time sense intra- and extracellular conditions and, thus, could be synthetically adapted to balance the biomass formation and overproduction of compounds by individual cells. Herein, we highlight advances in the designing and engineering approaches to intracellular biosensors. Then, the spatiotemporal properties of biosensors associated with the distribution of inducers are compared. Also discussed is the use of such biosensors to dynamically control the cellular metabolic flux. Such biosensors could achieve single-cell regulation or collective regulation goals, depending on whether or not the inducer distribution is only intracellular.

ARTICLE HISTORY
Received 9 November 2020
Revised 25 December 2021
Accepted 17 January 2022

KEYWORDS
Dynamic regulation; extracellular metabolite; intracellular metabolite; microdroplet; riboswitch; non-genetic variants; single-cell regulation

Introduction

Over recent decades, thousands of valuable compounds have been synthesized in microbial cell factories [1,2]. The primary goal of most metabolic engineering projects is to maximize metabolic flux to generate the highest product productivity. In this context, traditional, static metabolic engineering approaches, such as gene overexpression, knockouts, and repression, have shown substantial advantages by pumping cellular resources into product synthesis, thereby reducing product consumption [3]. To balance the metabolic flux of multigene metabolic pathways and avoid cellular resource exhaustion and the accumulation of intermediates, researchers have established innovative strategies, such as multi-module metabolic engineering [4], promoter engineering [5], RBS and 5′-UTR engineering [6,7], and protein scaffold strategies [8]. However, these strategies do not adapt well to changes in intra- and extracellular conditions, potentially impacting cell growth or productivity [9]. In contrast to traditional approaches, intracellular biosensor-based dynamic metabolic regulation balances tradeoffs between growth and productivity, using real-time monitoring and transducing the environmental signals into genetic expression signals [10,11]. Common examples are culture condition sensing [12,13], quorum sensing (QS) [14,15], and metabolite sensing [2,16,17] dynamic regulation approaches, which could, in real-time, balance the expression level of compound biosynthetic pathways and cell growth pathways according to changes in pH, temperature, cell density, and specific metabolites to maximize product production without impairing cell growth.

The intracellular biosensor is the most important part of dynamic metabolic regulation. Generally, the intracellular biosensor can be separated into three major classes: Förster resonance energy transfer (FRET)-based biosensors, transcription factor-based biosensors (TFBs), and riboswitch-based biosensors [18,19]. Among them, FRET-based biosensors are used mainly for high-

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throughput screening or cell imaging. However, there are no reports of their use in metabolic regulation [19]. In contrast, TFBs and riboswitch-based biosensors are commonly used for dynamic metabolic regulation. However, the responsive mechanisms of TFBs and riboswitch-based biosensors are significantly different. TFBs are protein-based biosensors. Environmental signals, such as temperature, light, chemicals in the medium, and specific metabolites, can induce a conformational change in the transcription factor (TF) and further activate or repress the transcription of target genes. Riboswitches are RNA-based biosensors, and only chemical signals can induce the conformational change of riboswitches to result in exposure or covering of the ribosome binding site (RBS) to activate or repress the translation of the target genes. Physical environmental signals, such as temperature and light, cannot induce such a conformational change. Intracellular biosensors are the signal transition center. Their performance is critical for their use in metabolic regulation. To investigate superior biosensor performance, studies have primarily focused on two aspects: (1) optimizing expression levels of TFs or target genes by promoter engineering, RBS engineering, and transcription factor binding site recombination; and (2) protein engineering to modify TF or DNA mutations to modify the riboswitch (Sections Enhancing TFB performance and Enhancing the performance of riboswitch-based biosensors) [20]. Using these optimization approaches, researchers have obtained an abundance of superior biosensors as a toolbox for dynamic metabolic regulation applications.

The current dynamic regulation reviews have focused mainly on the application advantages of the temporal level regulation of intracellular biosensors. These include using biosensors to monitor changes in pH, temperature, dissolved oxygen (DO), and specific metabolites in whole fermentation periods to allocate metabolic flux between cell growth and compound biosynthesis [21–23]. However, the fermentation systems generate significant heterogeneity of non-genetic variants, which include high- and low-performing cells [24]. Furthermore, in these fermentations, young and aged cells coexist in both exponential and stationary phases [25–27]. How dynamic regulation works on these significantly heterogenic cells with a spatial regulation perspective has been seldom discussed. As we know, the precise regulation of metabolic flux, according to the specific cellular state of individual cells, could significantly improve cell net productivity [28]. In reality, the time and spatial scope of inducers (temperature, DO, light, nutrients, and specific metabolites) directly impact the spatiotemporal properties of dynamic regulation.

Herein, we defined and separated the inducers into three categories depending on their spatiotemporal properties: (1) "environmental inducers," which exist everywhere in fermentation systems and include physical and chemical culture conditions, such as pH, temperature, DO, light, and nutrients (Sections Physical environment-response biosensor-based dynamic regulation and Chemical environment-response biosensor-based dynamic regulation); (2) "individual inducers," which exist only intracellularly and include: ATP, NADP(H), NAD(H), and malonyl-CoA (Section Intracellular metabolite-response biosensor-based dynamic regulation); and (3) "extracellular metabolite inducers," which are generated by cells, and most are secreted by cells, such as acetic acid, N-acyl homoserine lactones (AHLs), and aryl hydrocarbon receptor-interacting proteins (AIPs) (Section Extracellular metabolite-response biosensor-based dynamic regulation). We conclude with a discussion on the advantages and disadvantages of these three inducer types for mediating dynamic regulation, with a focus on critical factors of current and future metabolic engineering efforts.

Engineering strategies and the performance of intracellular biosensors

Intracellular biosensors innately serve as regulatory centers for dynamic regulation networks. Therefore, understanding and engineering their induction characteristics is crucial for metabolic flux redirection applications. Herein, we reviewed current strategies that enhance the performance of TF- and riboswitch-based biosensors to guide in designing efficient biosensors.

Enhancing TFB performance

Generally, response TFB models are classified into two categories based on the type of TFs: (1) activator-regulated models whereby transcription occurs when the activator is activated and binds to weak promoter regions, thus activating target gene expression [29], and (2) repressor-regulated models whereby transcription is repressed when the repressor is activated and binds to strong promoter regions, thus repressing target gene expression (Figure 1(A)) [30]. These intricately functioning biosensors exist widely in microbe genomes. Hence, genome mining by transcriptome and metabolism analysis is a promising approach to developing new biosensors. A comparative analysis of the transcriptome of microbes with and without induction

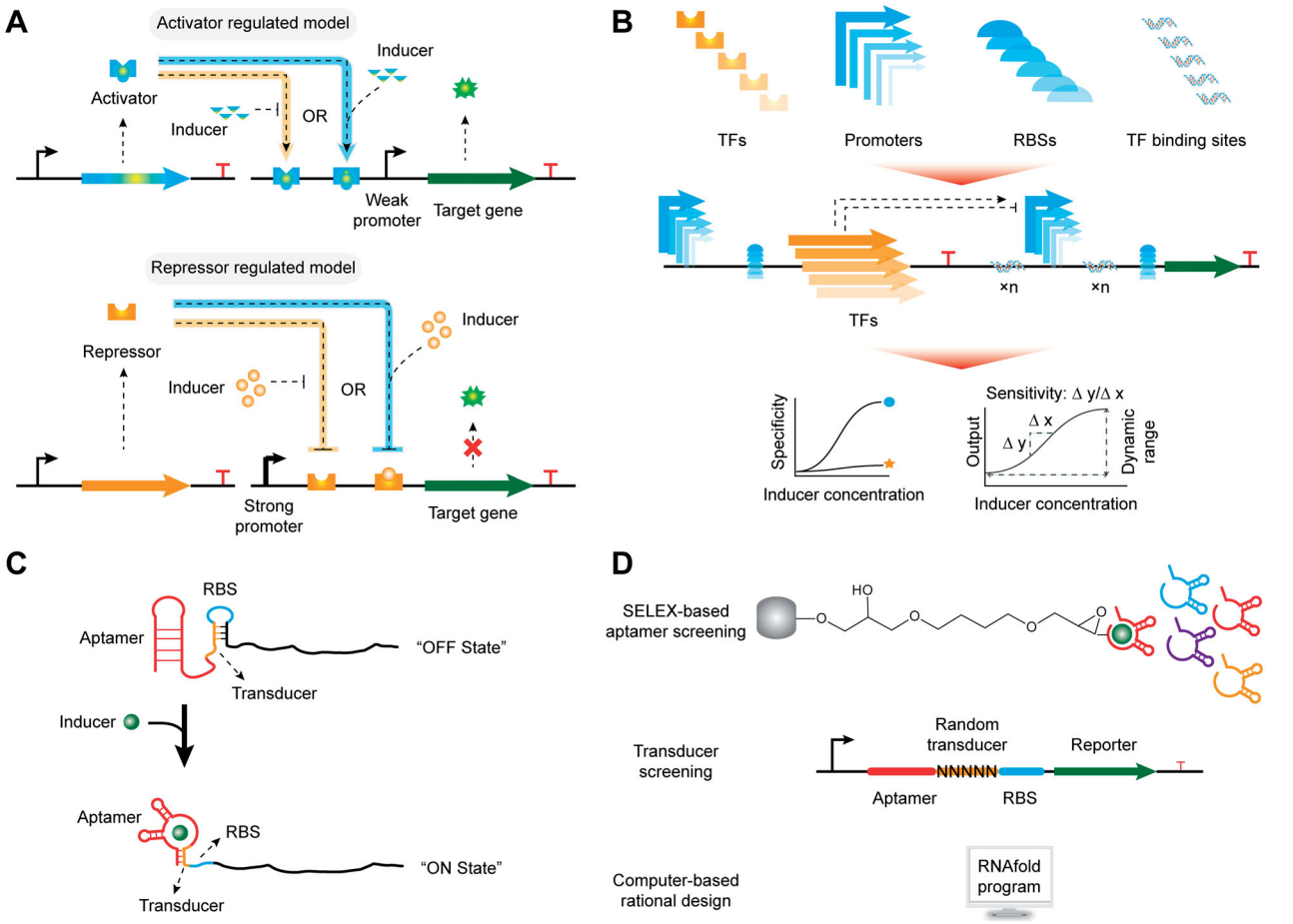

Figure 1. Regulatory mechanisms and engineering strategies for intracellular biosensors. (A) Biosensor response mechanisms of the activator and repressor regulated model. Blunt end arrows represent repression. Arrows represent activation. (B) Commonly used TFB engineering strategies. (C) Response mechanisms of riboswitch-based biosensors. (D) Riboswitch-based biosensor engineering strategies.

by specific inducers will often expose the expression of significantly different genes that could directly identify demanded TFs and inducible promoters to constitute biosensors. In this manner, some novel biosensors were screened, such as 1-butanol [31] and 3-dehydroshikimate-responsive biosensors [32]. On the other hand, the degradation pathway of some compounds is often regulated by TFs that respond to degradation intermediates [33]. In this scenario, Sun et al. found a TF *saro_0803*, which encodes a putative transcriptional regulator that could respond to resveratrol. This was found by analyzing resveratrol degradation enzymes from the genome of *Novosphingobium aromaticivorans* DSM 12444 [33].

All TFBs contains two functional elements, the TF, and the corresponding promoter region that senses and responds to induced TFs, and regulates downstream gene expression. In this regard, strategies that enhance TFB performance have focused on engineering TFs and promoter regions (Figure 1(B)) [30]. In terms of TF engineering, directed evolution-based protein engineering is the most commonly used strategy to evolve TFs. The generated mutations possibly could change the conformation of TFs and further influence the affinity with RNA polymerase, thus resulting in the reversed response model of TFBs. As an example, using an error-prone PCR-based directed evolution strategy, two novel QS switches—an "enhanced" repression/derepression repressor (eLsrR) and a "reversed" repression/derepression function activator (aLsrR) were obtained [34]. Analyzing the protein structure of eLsrR and aLsrR showed that conformational change is the most likely reason for the reversed response model. Moreover, the inducer specificity of TFBs could be altered by modifying the structure of the inducer binding pocket [35,36]. Amino acid residue replacement at the inducer binding pocket could alter its size and structure and further change the interaction with different inducers, resulting in a specificity change. In this scenario, L-arabinose response regulatory protein (AraC) was mutated and generated mutants that could activate transcription in response to L-arabinose-like

molecules, such as triacetic acid lactone [37], mevalonate [38], D-arabinose [39], and ectoine [40]. Although these directed evolution strategies have elicited significant advancements in TFB engineering, the approaches generate vast numbers of unfunctional or even negative mutants, which increase screening workloads.

All TFs consist of a DNA-binding domain (DBD) and ligand-binding domain (LBD). Hence, a combination of different DBDs and LBDs should generate novel functional TFs. Inducers binding with LBDs trigger allosteric effects in TFs, resulting in conformational DBD changes that bind or remove them from TF binding site regions. In this context, the most important challenge is how to achieve correct allosteric effects for domain swapping. Recently, Juárez et al. attempted to explore the assembly approach of DBDs and LBDs to obtain TFBs with the desired performance [41]. In doing so, these authors identified and analyzed the best combinatorial TFBs from a library generated by enhanced ligase cycling/chain reactions to assemble: 15 DBDs, a series of linkers, and 15 LBDs. They observed more than 3-fold expression differences when inducers were present or absent. This "chimeragenesis" method also facilitates the creation of novel biosensors. Inspired by this method, directed evolution only focused on LBDs as a promising strategy to enhance TFB performance. In this manner, Snoek et al. demonstrated a 15-, 5-, 40-, and 3.9-fold improvement in dynamic range (fold change in gene expression between inducer presence and absence), adipic acid specificity, detection range, and functional inversion of the *Acinetobacter sp.* BenM-based biosensor (cis-muconic acid-inducible), respectively [42]. Furthermore, crystal structure-based protein design is another rational design strategy to alter inducer specificity. As an example, Taylor et al. docked: fucose, lactitol, and sucralose into the ligand-binding pocket of LacI, to analyze key residues, and performed virtual mutation of amino acids [43]. After screening, the dynamic range of LacI mutants improved 10.5-, 7.1-, and 22-fold for fucose, lactitol, and sucralose, respectively. However, structure-based rational mutagenesis has been restricted by limited crystal structures. Hence, computational modeling-based protein rational design has attracted increasing attention. As an example, the inducer specificity of the *Acinetobacter* regulatory proteins (PobR) that can specifically be induced by 4-hydroxy benzoate but not 3,4-dihydroxy benzoate have been rationally engineered [44]. The authors applied homologous modeling and ligand-docking to identify amino acids that interacted with 4-hydroxy benzoate. After saturation mutagenesis of the 16 chosen amino acids and a fluorescence-activated cell sorting-based screening approach, mutants were produced with a 30-fold improvement in dynamic range with 3,4-dihydroxy benzoate induction. Taken together, directed evolution, domain swapping, and computational modeling are powerful TF engineering strategies. They enhance specificity and correlations between inducer concentrations and output signals.

A parallel approach to enhancing TFB performance includes promoter region engineering strategies. The core promoter and RBS have been well-investigated as engineering targets (Figure 1(B)). The core promoter controls the transcription level of TFs and reporters that are closely related to the: detection range, dynamic range, and leakage expression level. To investigate the influence of the core promoter on the dynamic range, the performance of TFB with various combinations of −10 and −35 boxes was systemically analyzed [45]. In doing so, Chen et al. validated that the equilibrium constant of σ^{70} binding to the core promoter was positively correlated with leakage and induction activity. Furthermore, Ding et al. [46] found that RBS also exhibited a significant influence on the dynamic range by controlling the protein's initial translation rates. The high protein translation rates generate unfolded or misfolded proteins, influencing the folding states of both the TF and reporter, thus regulating the dynamic range of TFBs. To precisely design TFBs, the authors established a deep learning-based model to predict the dynamic range with a given RBS input and achieved 72.2% prediction accuracy.

Enhancing the performance of riboswitch-based biosensors

Due to the TF translation process in signal transmission, TFBs experience a long response time that usually results in regulation delay. Hence, RNA-based biosensors are superior in this aspect. Riboswitches are small ligand-responsive regulators that control downstream gene expression by releasing or generating secondary structures at 5′-ends of mRNAs when inducers are present or absent, respectively. Altered conformations usually result in blocked translation, premature transcription termination, or changes in mRNA decay rates [47]. Riboswitches consist of an aptamer and a transducer that function as specific binding inducers and transit signals from input to output, respectively (Figure 1(C)) [48–50]. In recent decades, more than 40 different riboswitch classes have been established or discovered [51,52], of which RNA interactions with inducers are the most popular regulatory approaches. In this context, diverse riboswitch engineering

strategies have been established, including aptamer domain engineering, transducer domain engineering, and computer-based rational design strategies (Figure 1(D)) [53].

To derive a novel riboswitch function that is non-existent in nature, researchers focused on engineering aptamer domains [54]. The functional screening for *in vitro* aptamer functions uses systematic evolution of ligands by exponential enrichment (SELEX). This procedure is experimentally linked with an inducer immobilized on resins, such as epoxy-activated sepharose 6B, as a carrier to capture aptamers that bind with inducers from random sequence libraries (Figure 1(D)) [55]. However, to construct a functional riboswitch *in vivo*, a functional transducer domain must be generated to correlate inducer concentrations and gene expression states. To this end, a nucleotide random library was usually used as a transducer, to optimize the performance of a target sensor. To develop a naringenin riboswitch biosensor, Jang et al. screened an optimum transducer from a 10 nt random library, generating a 2.91-fold output signal increase upon 200 mg/L naringenin [55]. Although riboswitches derived from random sequences achieved significance: success, time, and labor were challenging issues for high-throughput screening endeavors [56]. Thus, computer-based rational design strategies have been successfully instigated and used to evaluate changes in folding energies [57]. For example, Domin et al. designed tetracycline and streptomycin riboswitches based on their secondary structures and free energy values, predicted by the RNAfold program of the Vienna RNA package [58,59]. A similar approach using binding energies was conducted using multi-analyte aptamers for *in vitro* aptamer function [60].

Spatiotemporal properties of intracellular biosensors

Despite significant engineering efforts for intracellular biosensors, few studies have concluded or discussed their spatiotemporal responses. Generally, inducer spatiotemporal distribution states directly influence the induction mode (Figure 2). The temporal properties of biosensors depend mainly on the real-time changes of inducers. According to differences in inducer spatial

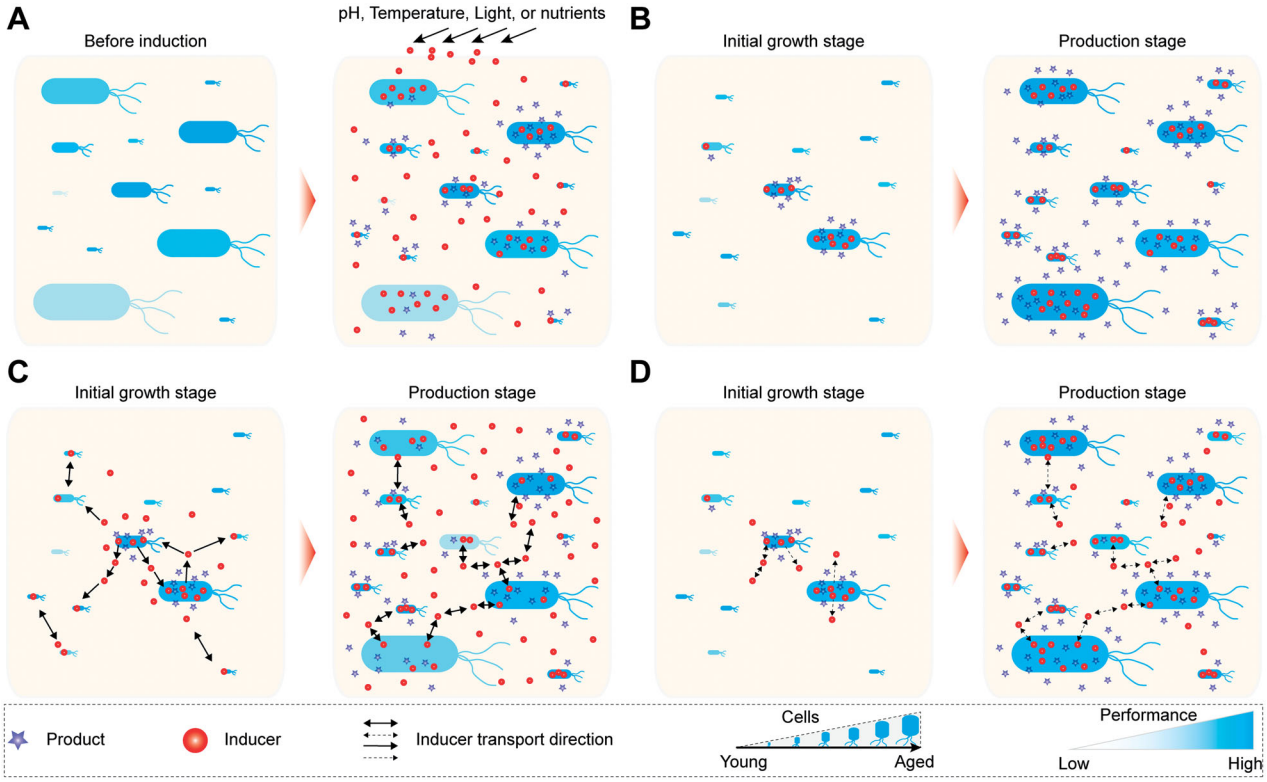

Figure 2. How the spatiotemporal distribution of inducers affects the dynamic regulation mode of the intracellular biosensors. (A) All cells are induced in environment-response dynamic regulation strategies when inducers are present. (B) Cells are induced based on their individual states using intracellular metabolite-response dynamic regulation strategies. There is no cross-talk between different cells, and all cells achieve high performance. (C) Cross-talk between different cells involved in extracellular metabolite-response dynamic regulation strategies. Only certain cells maintain high performance. (D) Limited cross-talk between different cells, when only a small proportion of inducers are transported out of cells.

ranges, we aggregated dynamic regulation into three categories: (1) environment-response modes where induction factors (environmental inducers) exist in all areas of the fermentation system (Figure 2(A)), (2) intracellular metabolite-response modes where induction factors (individual inducers) exist only intracellularly (Figure 2(B)), and (3) extracellular metabolite-response modes where induction factors (extracellular metabolite inducers) are extracellularly based (Figure 2(C)). In this context, regulatory circuits can be controlled at the spatiotemporal level by changes in the global environment and intra- and extracellular metabolites.

However, although environment-response dynamic regulation achieves real-time gene expression control by monitoring global environment changes, such as temperature, pH, nutrient supplementation, and light induction, it still suffers from a "collective regulation problem." Specifically, this mode of control regulates and synchronizes cellular gene expression at a singular, special induction point, without considering differences between intracellular single-cell states under different conditions (Figure 2(A)) [12,61]. In contrast to extracellular signals, certain metabolites, i.e., CoA thioesters, ATP, and NADP(H) only accumulate in the cytoplasm or other organelles. As such, intracellular metabolite-response dynamic regulation could fine-tune target gene expression by accounting for inducer concentrations under different growth conditions (Figure 2(B)). However, many small molecule metabolites are secreted outside the cell, resulting in extracellular metabolite-response or intra- and extracellular concomitant metabolites-response dynamic regulation (Figures 2(C,D)). In this context, cross-talk induction, amongst different cells, would result based on the transfer ratio of formation, uptake, and secretion. Taken together, the spatiotemporal range of environment and metabolite inducers not only control the expression timing of target genes but also influence individual expression states within populations.

Environment-response dynamic regulation

Fermentation optimization often involves condition alteration, such as pH, temperature, DO, and nutrients, to enhance the metabolic flux of target compounds. However, empirical fermentation optimization often suffers unstable effects when medium or seed cultures vary. Likewise, these conditions exhibit spatial variations in large bioreactor systems, with cells transiting throughout different regions. Hence, constructing dynamic regulatory networks based on environment-response biosensors is a promising approach to these macroscopic changes [11]. Herein, we highlighted the use and advantages of physical and chemical environment-response dynamic regulation strategies.

Physical environment-response biosensor-based dynamic regulation

Temperature and light as "environmental inducers" could control the activity of biosensors by thermal and optical radiation, respectively. Thus, they could result in the environment-response dynamic regulation mode. Generally, temperature and light-responsive biosensors are the most commonly used regulatory factors for physical environment-dependent dynamic regulation networks. One particular temperature-response biosensor consists of a promoter P_R/P_L system, along with a temperature-sensitive repressor protein, CI857 from bacteriophage λ. This approach generates dynamic control of target gene expression in relation to temperature [62,63]. When the temperature is below 30 °C, CI857 binds to a P_R/P_L tandem promoter, blocking transcription. However, once the temperature exceeds 37 °C, CI857 rapidly becomes inactivated and transcription commences from P_R/P_L (Figure 3(A)). Based on this mechanism, Sun et al. constructed a non-glucose-utilizing strain by overexpressing a xylose transporter (*xylH*) in *E. coli* B0013-2020H whose glucose uptake and metabolism genes (*ptsG*, *manZ*, and *glk*) were knocked out for ethanol production from lignocellulosic hydrolysates [64] (Table 1). Using *E. coli* B0013-2020H as chassis, the authors recovered the expression of *ptsG* (essential for glucose uptake and metabolism) by P_R/P_L promoter. Thus, a "two-phase-two-temperature" fermentation strategy was established, which used xylose and glucose as carbon sources for cell growth and ethanol biosynthesis when temperatures shifted from low to high, respectively. Finally, after a 12 h fermentation, the ethanol titer improved by 1.42-fold when compared with the negative control (4.06 *vs.* 2.85 g/L/h). Such a temperature-based two-stage dynamic regulation strategy can achieve a balance between cell growth and biosynthesis. However, challenges still exist, i.e., expended extra energy and regulatory delays when the temperature changes. Thus, a less expensive and more sensitive dynamic regulatory strategy is required.

As alternatives, light-response biosensors can: remotely, instantaneously, and precisely regulate target gene expression. Such approaches can avoid the disadvantages of temperature-response dynamic regulation strategies. The TF of EL222-based biosensor can be activated by blue light and forms homodimers to promote P_{C120} promoter-controlled gene expression (Figure

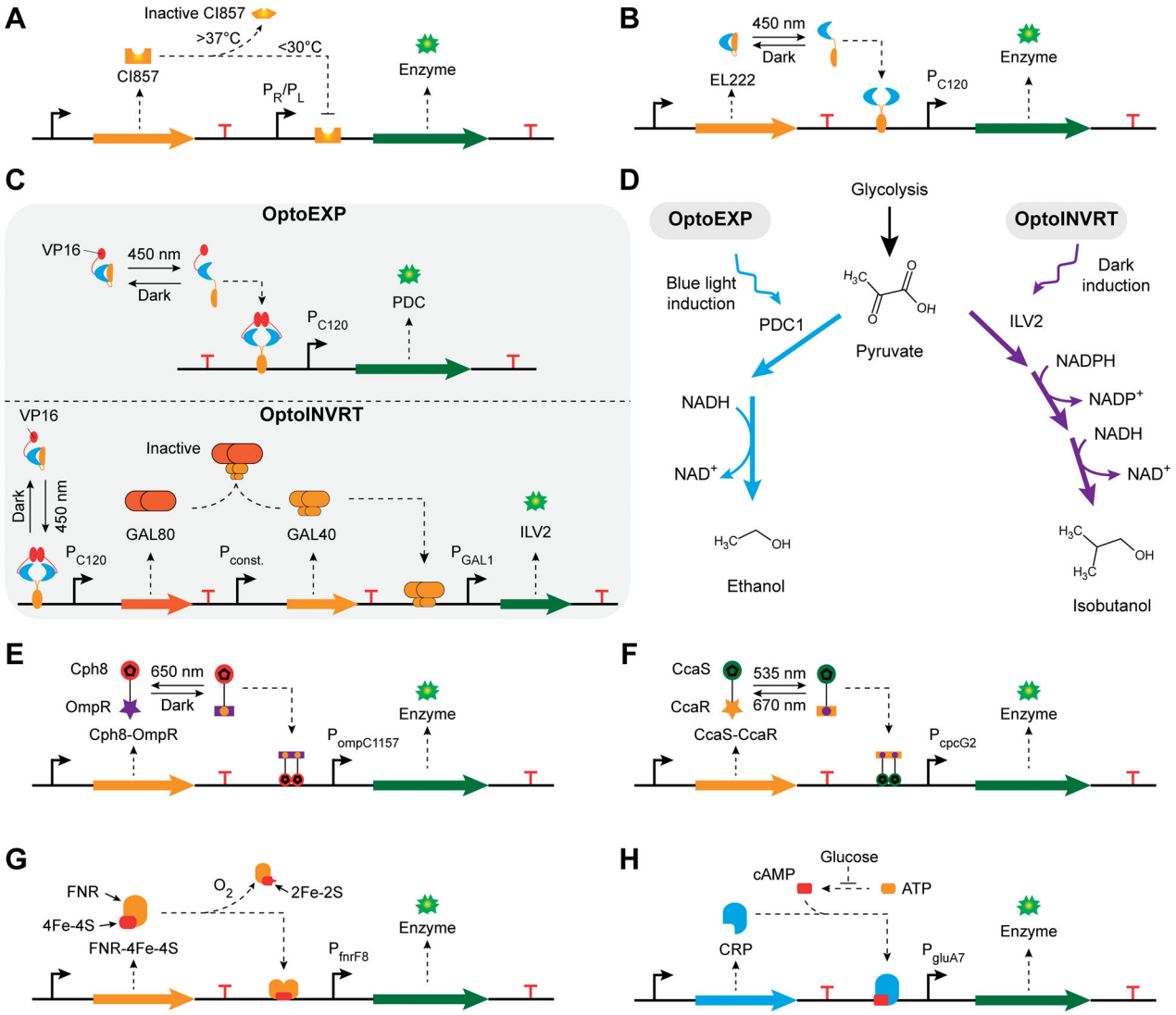

Figure 3. Dynamic regulation mechanisms of environmental-response biosensors. Regulation mechanisms of temperature-response biosensors (A) and blue light-response biosensors (B). (C) The dynamic regulation mechanisms of OptoEXP and OptoINVERT systems. (D) The application of OptoEXP and OptoINVERT systems in the biosynthesis of isobutanol, wherein PDC1 expression for ethanol production will complete the NAD^+ recycling and ATP generation to support cell growth. Regulation mechanisms of red light-response biosensors (E), green light-response biosensors (F), oxygen-response biosensors (G), and glucose-response biosensors (H). A pentagon represents phycocyanobilin. Yellow and purple circles in (E,F) represent phosphate groups. Blunt end arrows represent repression; Arrows represent activation.

3(B)). This TFB could be used to construct functionally opposite optogenetic gene expression systems, such as OptoEXP and OptoINVRT, to instantaneously and precisely balance cell growth and production [65]. In doing so, the OptoEXP system was controlled by VP16-EL222 (EL222 fused with a transcriptional activation domain VP16), which was activated by blue light, to turn on the expression of P_{C120} promoter-controlled pyruvate decarboxylase (PDC1). However, the OptoINVRT system combined VP16-EL222 with the yeast galactose (GAL) regulon (GAL4-GAL80) to promote the expression of P_{GAL1} promoter-controlled acetolactate synthase (ILV2) under dark conditions (Figure 3(C)). By using a periodic light-based two-phase fermentation strategy, the pyruvate was precisely allocated between ethanol and isobutanol synthetic pathways for balancing cell growth and production, resulting in 8.49 ± 0.31 g/L isobutanol and 2.38 ± 0.06 g/L 2-methyl-1-butanol (Figure 3(D) and Table 1) [65]. The other light-inducible biosensors, the Cph8-OmpR-based red light-inducible biosensor, and the CcaS-CcaR-based green light-inducible biosensor rely on the conformational transition of OmpR or CcaR between dephosphorylation and phosphorylation states [65,66]. Phycocyanobilin-ligated Cph8 or CcaS catalyzes this transition when induced by red or green light, respectively (Figures 3(E,F)). However, these

Table 1. Intracellular biosensors mediated dynamic regulation with different modes according to the spatiotemporal properties of the inducers.

Inducer category	Characteristics	Inducer	Biosensors*	Applications**	Results
Environment-response modes					
Environmental inducers	Environmental inducers spatially existed in all areas of the fermentation system. Thus, they could induce every cell in the system according to the induction dosage, ignoring the differences between individual cells.	Temperature	CI857∼P_R/P_L	Dynamically regulate the uptake of glucose in *E. coli* (⟺ *ptsG*⇑*xylH*Δ*manZ*Δ*glk*) for ethanol production.	2.85 g/L/h [64]
		Blue light	VP16-EL222∼P_{C120}; GAL4-GAL80∼P_{GAL1}	Dynamically regulate the flow direction of pyruvate in *S. cerevisiae* (⟺ *PDC1* ⟺ *ILV2*Δ*GAL80*Δ*PDC156*) for fusel alcohol biosynthesis.	10.87 g/L [65]
		Red, green, and blue light	Cph8-OmpR ∼ P_{λ}; CcaSR ∼ $P_{cpcG2-172}$; and YF1-FMN ∼ P_{PhlF}	Using this RGB system to control the expression of three sigma fragments (σ_{K1F}, σ_{CGG}, and σ_{T3}) to reduce the production of acetate in *E. coli* (⟺ *pta* ⟺ *ackA* ⟺ *poxB*)	Acetate accumulation reduced 50% [68]
		Blue light; temperature	paRC9∼P_{sgRNA}; tsRC9-P_{sgRNA}	Using the dCas9 variants (paRC9 and tsRC9) to repress the target gene expression by sgRNA, which was controlled by the promoter P_{sgRNA}.	3- and 5-fold repression achieved in light and 29 °C, respectively [61]
		pH	Haa1p ∼ P_{YGP1pr}	Producing lactic acid in *E. coli* (⟺ *ldhL*) under low pH environment.	10-Fold improvement [71]
		Oxygen	FNR ∼ P_{nar}	Dynamically regulate the synthetic pathways in *E. coli* (⟺ *ldhD*), *E. coli* (⟺ *ilvBN* ⟺ *aldB* ⟺ *bdh1*), and *E. coli* (⟺ *dhaB1B2B3* ⟺ *yqhD* ⟺ *gdrA* ⟺ *gdrB*) to produce 2,3-butanediol, D-lactate, and 1,3-propanediol, respectively.	The titer reached 48.0, 113.12, 15.8 g/L, respectively [77]
		Glucose	cAMP-CRP ∼ P_{tetOp}	Balancing cell growth and polyhydroxybutyrate production in *E. coli* (⟺ *phaECAB*).	Growth 5-fold faster with comparable titers to control [86]
		Xylose	XylR-VPRH ∼ P_{adh1}	Dynamically regulate the xylose utilization pathway in *Y. lipolytica* (⟺ XDH ⟺ XKS) to grow in a medium with xylose as the sole carbon source.	2-fold improvement in growth [88]
Intracellular metabolite-response modes					
Individual inducers	Individual inducers exist only intracellularly. Thus, they could induce cells in the system according to their individual states.	Malonyl-CoA	FapR ∼ P_{GAP}/$P_{T7\text{-}fapO}$	Establishing an oscillator to dynamically regulate the fatty acid production in *E. coli* (⟺ *accADBC* ⟺ *fabADGI* ⟺ *tesA*)	15.7-fold improvement in fatty acid titer [95,97]
		Acyl-CoA	FadR ∼ P_{fadBA}	Dynamically regulate the production of fatty acid ethyl ester in *E. coli* (⟺ *pdc* ⟺ *adhB* ⟺ *fadD* ⟺ *atfA*).	1.5 g/L [93]
		NADH	B-Rex ∼ $P_{ROP\text{-}PP}$	As a tool for high-throughput screening.	80% screening accuracy [102]
		(2*S*)-Naringenin	FdeR ∼ P_{fdeA}	Establishing a (2*S*)-naringenin-responsive amplifier to gradually elevate the repression level of malonyl-CoA consumption pathway (⟺ *acpHST*) in the fermentation process to produce (2*S*)-naringenin.	Combining the two systems obtained a 523.7 mg/L titer [16,129]
		p-Coumaric acid	PadR ∼ P_{PadC}	Dynamically regulate the malonyl-CoA formation pathway (⟺ *acs* ⟺ *ACC*) according to the accumulation level of *p*-coumaric acid to produce (2*S*)-naringenin.	
Extracellular metabolite-response modes					
Extracellular metabolite inducers	Extracellular metabolite inducers were secreted by cells. They existed spatially in all areas of the fermentation system. Thus, they generate cell-to-cell cross-talk, resulting in a collective induction phenomenon.	AHLs	EsaI-EsaR ∼ P_{esaS}	Coupling the two biosensors to repress cell growth and activate the synthetic pathway when the biomass reaches the threshold for the production of naringenin and salicylic acid.	463 μM naringenin and 520 mg/L salicylic acid [14]
		AHLs	LuxI-LuxR ∼ P_{luxI}		
		Acetic acid	NRI ∼ P_{glnAP2}	Controlling cell growth by coupling the dynamic regulation system of acetic acid biosynthesis.	249-fold induction when threshold reached [112]
		cis,*cis*-muconic acid	BenM ∼ $P_{CYC1\text{-}BenO}$	As a sensor-selector to selectively enrich for best-producing variants out of a large library of muconic acid production strains.	2 g/L [118]

*Include TFBs and riboswitch biosensors. The TF ∼ promoter pairs of TFBs were demonstrated.
** ⟺ represents the expression of genes controlled by relative biosensor; ⇑ represents the genes overexpressed.

biosensors have issues, such as bulky DNA encoding, leaky expression, and low dynamic ranges. To overcome these limitations, Schmidl et al. attempted to optimize the genetic expression levels of both Cph8-OmpR and CcaS-CcaR systems, resulting in a 72-fold reduction in leaky expression and a 117-fold enhancement in the dynamic range [67].

Owing to the excellent orthogonality of red, green, and blue light-responsive biosensors, more versatile genetic circuits can be designed to achieve the various engineering requirements. In this regard, a red, green, and blue (RGB) light-controlled system was established for triple-layered, dynamic, regulating, target genes [68]. In doing so, the phage RNA polymerase was split into a constitutively expressed non-active "core" fragment and a light induction "sigma" fragment. An active RNA polymerase can be generated only when "core" and "sigma" fragments are combined. In this scenario, using red, green, and blue light biosensors to control the expression of three sigma fragments (σ_{K1F}, σ_{CGG}, and σ_{T3}), the activity of RNA polymerases could be dynamically regulated, which resulted in the control of the strength of the sigma-specific promoters P_{K1F}, P_{CGG}, and P_{T3}. As a proof-of-concept, Fernandez-Rodriguez et al. used this RGB system to redirect the metabolic flux of acetate biosynthesis by dynamically regulating the expression of sgRNAs (*poxB*, *ackA*, and *pta*) to repress the acetate production pathway. This exhibited good orthogonality properties (Table 1) [68]. However, these light-response dynamic regulation strategies strictly rely on the induction of specific promoters by chromophore-ligated two-component regulation proteins, which restrict the application flexibility. To obtain biosensors that could regulate arbitrary given promoters, researchers speculated that *Rhodobacter sphaeroides*' blue light-sensing domain (*Rs*LOV) could confer light sensitivity ability onto an arbitrary TF protein by constructing a fusion protein. As a proof-of-concept, Richter et al. hybridized the Cas9 and the *Rs*LOV to derive arbitrary functional regulators [61]. In doing so, the authors inserted *Rs*LOV into the 234 functional sites of Cas9, generating an insertion library to screen the desired phenotype variants. Finally, two inducible Cas9 variants, paRC9 (blue light-inducible) and tsRC9 (temperature inducible) were screened and exhibited hybrid functions.

Overall, compared with the temperature-response dynamic regulation, light-response dynamic regulation exhibits significant advantages, such as low fermentation costs and instantaneous and spatiotemporal control of gene expression. However, turbid fermentation fluids lead to inhomogeneous induction, and fickle extra- and intracellular conditions significantly disturb the induction effects of light-response dynamic regulation. In this regard, chemical environment-response, biosensor-based, dynamic regulation should be an effective strategy for solving these challenges.

Chemical environment-response biosensor-based dynamic regulation

Fermentation is always accompanied by: fickle condition changes, especially nutrient concentrations, DO, and pH. Thus, it is important to establish more flexible regulation strategies, independent of artificial induction, that dynamically regulate target gene expression, based on changes in fermentation conditions. Specifically, it is difficult and expensive to maintain suitable pH conditions for optimal balanced cell growth, and organic acid production. Hence, the establishment of pH-response biosensor-based dynamic regulation networksis necessary. The low pH activated TF, Haa1p, and its promoter P_{YGP1pr} were identified from *Saccharomyces cerevisiae* [69–71]. As an example of applications, Rajkumar et al. used P_{YGP1pr} to dynamically regulate the expression of the lactate dehydrogenase gene (*ldhL*) under low pH conditions to produce lactic acid. They achieved a 10-fold increase in titer (7.9 *vs.* 0.72 g/L) (Table 1) [71]. In contrast to the low extracellular pH, intracellular pH was maintained at neutral with mild changes. Extracellular undissociated lactic acid was transported into the cytoplasm, reducing the intracellular pH and resulting in activation of the P_{YGP1pr} promoter [72]. Besides lactic acid, other acids also could change the intracellular pH by being transported into the cytoplasm, achieving the goal of pH-based dynamic regulation. Considering the spatiotemporal properties of undissociated acids, such as lactic acid and acetic acid, pH-based biosensors could simultaneously and dynamically regulate the gene expression of all living cells in the same fermentation system, achieving the goal of collective regulation. On the other hand, stable intracellular pH is an essential condition for cellular growth and production. The strains with high acid tolerance often can maintain neutral intracellular pH when grown in low pH conditions. With this background, a continuous directed evolution system could be designed based on a set of pH-response riboswitch-based biosensors for screening acid tolerance mutants [73]. In doing so, such pH-based riboswitches were used to control the expression of a mutator (*ednaQ* that could reduce mismatch repair capacity, and generate a 106-fold enhancement in mutation rates in genome replication) according to differential intracellular pH

conditions [74]. Hence, under acid stress conditions, superior mutants maintained neutral intracellular pH conditions and reduced *ednaQ* expression. However, the acidic intracellular pH of negative mutants promoted *ednaQ* expression and, thus, increased mutation rates. Using this biosensor, a broad spectrum of microbial cell factories can be evolved to enhance acid tolerance.

Dissolved oxygen spatially exists everywhere in the fermentation system and is transported into the intracellular space by free diffusion. Thus, DO is another "environmental inducer" that could result in the environment-response dynamic regulation mode. DO is also a critical factor that influences fermentation productivity, especially under anaerobic or micro-anaerobic conditions. The wild-type *E. coli* P_{nar} promoter can be activated under both anaerobic and nitrate conditions. To derive DO-response promoters, Han et al. [75] and Lee et al. [76] engineered −10/−35 boxes from P_{nar} promoters by site-directed mutagenesis to generate variants that could be activated under anaerobic conditions without nitrate. Using the P_{nar} promoter to express the 1,3-propanediol synthetic pathway genes (*dhaB1B2B3*, *yqhD*, *gdrA*, and *gdrB*) in *E. coli* resulted in titers of 48.0 ± 8.48, 113.12 ± 2.37, and 15.8 ± 0.62 g/L for 2,3-butanediol, D-lactate, and 1,3-propanediol, respectively, in fed-batch and low oxygen conditions (Table 1) [77]. To date, the most studied DO-response biosensor is the fumarate and nitrate reductase (FNR) transcriptional activator-dependent biosensor. FNR activity is regulated by the transition between 4Fe-4S and 2Fe-2S clusters, according to oxygen levels (Figure 3(G)) [78]. Accordingly, more than 103 operons are regulated by FNR in the literature, where all promoters carry an FNR binding site; i.e., TTGA(T/C)NNNN(A/G)TCAA [79]. As synthetic biology parts, many synthetic or native DO-response promoters with superior performance have been reported (e.g., the synthetic promoter of P_{fnrF8} [80], the native promoters of dimethyl sulfoxide reductase (P_{dmsA}), pyruvate formate-lyase (P_{pfl}), nitrite reductase (P_{nirB}), ethanol dehydrogenase (P_{adhE}), from *E. coli* [81,82], and the hemoglobin promoter (P_{vgb}) from *Vitreoscilla* [83]).

During fermentation, residual carbon sources, especially sugars, typically control metabolic flux and cellular growth stages. However, traditional fermentation optimization is difficult for real-time measuring and regulating metabolic flux, based on sugar concentrations. To address this issue, a series of sugar-response biosensors were developed, such as the cAMP-CRP-dependent glucose-response biosensor, and the XylR dependent xylose-response biosensor [84,85]. Generally, glucose represses the biosynthesis of the global regulator (cAMP which combines with CRP), generating a cAMP-CRP complex to activate target gene expression (Figure 3(H)). Combining cAMP-CRP biosensors with the polyhydroxybutyrate biosynthetic pathway (*phaECAB*) impaired cell growth, and the cell growth and production phases were autonomously decoupled according to the concentration of glucose. Finally, this dynamic regulation system-generated 5-fold faster growth rates and comparable titers to constitutively expressed strains (Table 1) [86]. Glucose as a manually added carbon source existed spatially anywhere in the fermentation system. It was transported into the intracellular space by facilitated diffusion assisted by glucose transporters, resulting in an environment-response dynamic regulation mode. Differently, xylose was transported into the intracellular space by facilitated diffusion or active transport, depending on the type of xylose transporter. Xylose is one of the main contents of lignocellulosic biomass, the most abundant renewable and non-edible raw material in the world. However, it is not used by microbes since the expression of the xylose catabolism pathway is inhibited by glucose [87]. To facilitate the consumption of xylose, Wei et al. attempted to establish a xylose inducible biosensor in yeast by combing the *E. coli* activator XylR, the eukaryotic universal activation domain VPRH, and the xylO hybrid promoter to control the gene expression of xylose catabolism in response to xylose concentrations [88]. When compared with the constitutive expression of the xylose consumption pathways (XDH and XKS), biomass was more than 2-fold higher using this biosensor, with xylose as the sole carbon source in the YPD medium.

Overall, environment-response biosensors mediate dynamic regulation and avoid the complicated fermentation online controlling and achieve the goal of auto adaptation to the environment changes. On the other hand, while the temporal regulation of environment-response biosensors is outstanding, spatial regulation suffers a "collective regulation problem" which could not distinguish, and niche, targeting individual cells according to their growth states. In this regard, metabolite-response dynamic regulation plays a crucial role in single-cell spatiotemporal regulation.

Metabolite-response dynamic regulation

Fermentation systems in a bioreactor or shake flask may be regarded as promiscuous groups where various cells with different states coexist, including young and aged cells, high and low-performance cells, and high

and low burden cells. Thus, the biggest challenge is how to regulate individual cells based on their unique states, to achieve the highest cell productivity in all fermentation phases. Herein, we focus on current strategies that dynamically regulate the metabolic flux of single cells at spatial and temporal levels.

Intracellular metabolite-response biosensor-based dynamic regulation

Generally, metabolites that exist only in the cytosol or within an organelle are regarded as intracellular metabolites or individual inducers, e.g., CoA and its thioesters, ATP, NAD(H), and NADP(H). Concentrations of these metabolites are sensitive to extra- and intra-cellular environment fluctuations which can, in turn, influence: cellular redox states, energy states, and metabolic flux distributions [89–91]. These properties endow unique metabolic states onto individual cells and avoid regulation cross-talk in fermentation processes, unlike extracellular signaling. In this regard, productivity may be improved by dynamically regulating cell metabolic flux, according to the state and concentration of individual intracellular metabolites (Figure 2(B)).

Intracellular metabolite-response biosensors may perform as single-cell sensing and regulation centers. CoA thioesters and NADH/NAD^+ based dynamic regulation are two well-studied areas [92]. FapR and FadR are usually coupled with their response promoters to generate malonyl-CoA and acyl-CoA response biosensors, respectively. Acyl-CoAs not only usually acts as carbon chain extension units for complicated compound formation, but it also influences intracellular CoA balancing and protein CoAlation levels [2,89,90]. As an example to balance tradeoffs between acyl-CoA accumulation and compound formation, Zhang et al. established a dynamic sensor-regulator system (DSRS) to control the: biosynthesis, degradation, and transport of fatty acids using FadR-based TFBs [93]. When acyl-CoA accumulates, FadR binds to it and is released from P_{fadBA} to switch on target gene expression (Table 1) (Figure 4(A)). The established DSRS stably produces 1.5 g/L biodiesel. CoA thioester not only participates in the carbon chain extension but is also closely related to cell growth. Malonyl-CoA is used mainly to synthesize fatty acids for cell membrane formation and only accumulates at very low concentrations in some prokaryotes, limiting its ability for high titer production [94–96]. To accumulate more malonyl-CoA for production with no negative impact on cell growth, Xu et al. [95] and Wen et al. [97] attempted to use FapR to establish oscillators in *E. coli* and *Komagataella phaffii*. The common feature of both oscillators was upregulation of the malonyl-CoA biosynthetic pathway or downregulation of the malonyl-CoA consumption pathway when malonyl-CoA levels were reduced or accumulated, respectively (Table 1).

Another important intracellular metabolite is NAD^+, which plays a critical role in maintaining cellular redox and energy states. The conversion of NADH to NAD^+ occurs during aerobic respiration processes in the cytosol or mitochondria, depending on cell type (i.e., prokaryote or eukaryote). In prokaryotes, Rex is a common regulator used for the dynamic regulation of target gene expression [98–100]. Naturally occurring Rex is found in Gram-positive bacteria, such as *Bacillus subtilis*, which sense the poise of NADH/NAD^+. Rex is then bound or released from its operator sites, to modulate transcription levels of respiratory chain genes [101]. High NADH concentrations can inhibit the DNA-binding activities of Rex. As such, to obtain high NADH mutants, Liu et al. attempted to establish a Rex-based NADH/NAD^+ ratiometric biosensor in *E. coli* for real-time monitoring of redox states and high-throughput screening of engineered strains, resulting in 80% screening accuracy by fluorescence-activated cell sorting (Table 1) [102]. In eukaryotes, NAD(H) oxidation and reduction mainly occur in the mitochondria, thus a series of mitochondria-targeted TFBs have been recently established [103–105]. By ligating to mitochondria-specific signal sequences, TFs were targeted in the mitochondria to monitor redox states [106]. To date, most mitochondrial-targeted NADH/NAD^+ biosensors have been used for redox state detection or cell imaging. No metabolic engineering applications have been reported [107,108]. However, we believe the sophisticated dynamic regulation networks targeted at mitochondria will be established shortly.

Due to the lack of high-efficiency transporters, some liposoluble metabolites are usually difficult to transport to cell exteriors, exist mainly intracellularly, and are classified as intracellular metabolites. Recently, we compared the intracellular and extracellular concentrations of (2*S*)-naringenin and *p*-coumaric acid, finding that the intracellular levels were at least 58.1- and 10.2-fold higher than the extracellular content across all growth phases, respectively [16]. In this scenario, (2*S*)-naringenin and *p*-coumaric acid are considered as individual inducers that can achieve single-cell dynamic regulation by utilizing the relative biosensors as a means to maximize (2*S*)-naringenin production. To do so, we designed a multi-layered dynamic regulation network (NCOMB DRN) enabling single cells to concurrently regulate: *acpH*, *acpS*, *acpT*, *acs*, and *ACC* in malonyl-CoA catabolic

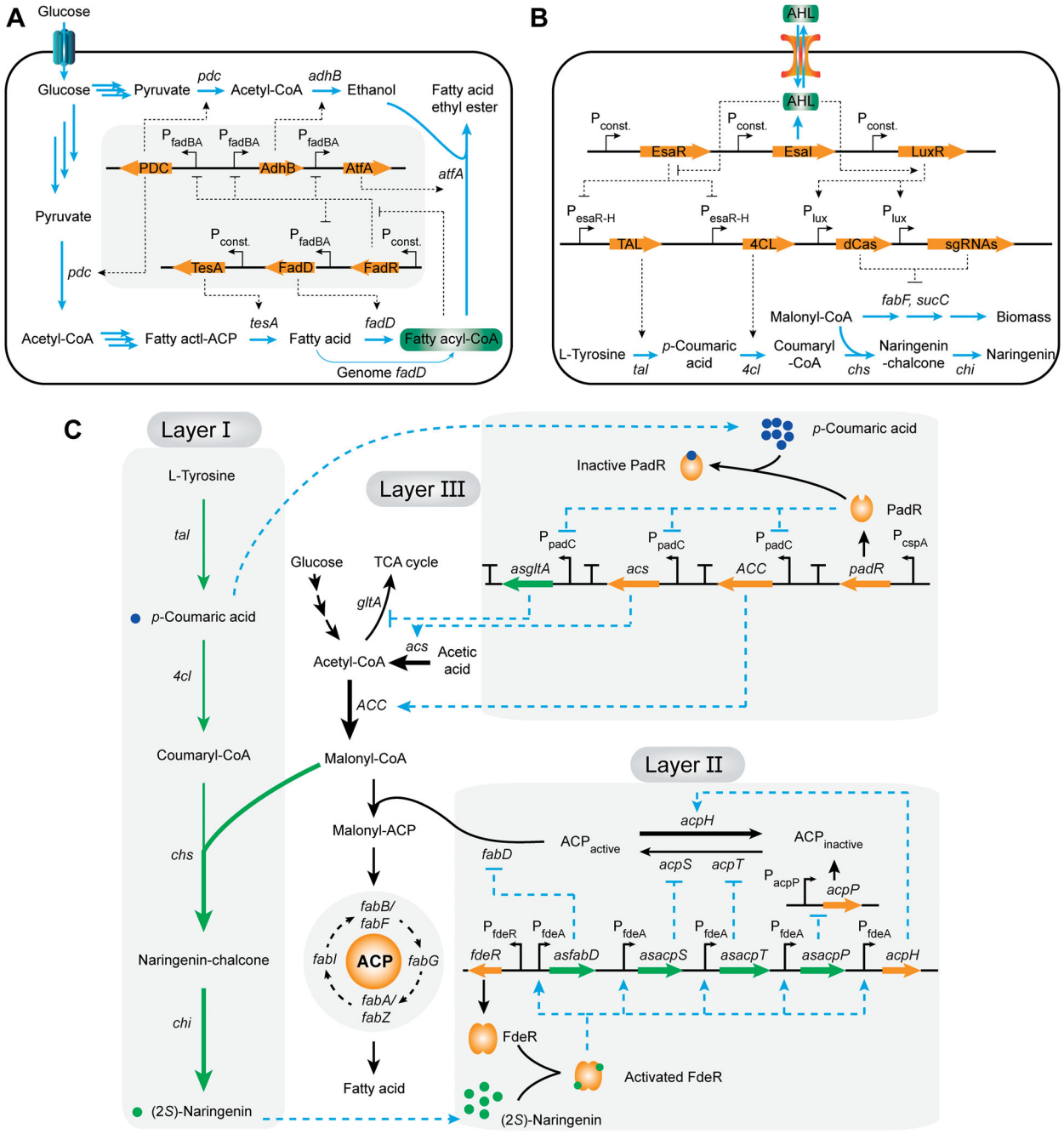

Figure 4. Dynamic regulation mechanisms of metabolite-response biosensors. (A) Dynamic regulation mechanisms of DSRS. As an "individual inducer," fatty acyl-CoA cannot be transported out of cells, achieving single-cell regulation. (B) Dynamic mechanism of QS-based bifunctional dynamic regulation. As an "extracellular metabolite inducer," AHL is transported into or out of cells, achieving collective regulation. (C) Dynamic regulation pathways of the NCOMB DRN [16]. Layer I: Constitutively expressed (2*S*)-naringenin biosynthesis pathway; Layer II: (2*S*)-Naringenin-responsive fatty acid repression module. Layer I and II could constitute a (2*S*)-naringenin-responsive amplifier; Layer III: *p*-Coumaric acid-responsive malonyl-CoA enhancing module. Blunt end arrows represent repression, and arrows represent activation.

and anabolic pathways (Figure 4(C)). After a series of optimization processes, (2*S*)-naringenin titer improved 8.7-fold (523.7 mg/L) with a concomitant 20% increase in cell growth compared to the static strain engineering approach (Table 1) [16]. Hence, using these un-transportable intracellular metabolites as individual inducers is a promising approach to achieving single-cell level dynamic regulation. Furthermore, we believe that engineering the transporter of intracellular metabolites to modify its transport efficiency and further change the spatial distribution would switch the dynamic regulation modes between "intracellular metabolite-response mode" and "extracellular metabolite-response mode."

Extracellular metabolite-response biosensor-based dynamic regulation

Some metabolites are transported to cell exteriors after formation and only kept at very low levels in cells. Such metabolites are defined as extracellular metabolites in this review. These secreted metabolites can enter other cells to generate cross-talk (Figure 2(C)) [109]. Two key examples include the applications of acetic acid and QS-response biosensor-mediated dynamic regulation. In this section, we highlight dynamic regulation strategies and regulation mechanisms in these areas.

Acetic acid is a product of incomplete glucose oxidation, the accumulation of which significantly disturbs intracellular pH. Thus, most acetic acid is secreted out [110]. Acetic acid is catalyzed by acetate kinase (*ack*), to generate acetyl phosphate. This acts as a global signal that regulates target gene expression by donating phosphoryl groups to TFs [111]. Bulter et al. observed that acetyl phosphate phosphorylated the TF, NRI to facilitate P_{glnAP2} promoter expression [112]. Given that *pta* and *poxB* are the main genes in acetic acid production, to reduce acetate accumulation Moser et al. attempted to establish an acetic acid, oxygen, and glucose-response dynamic regulation network, which automatically repressed *pta* and *poxB* expression at early and late growth stages. As a result, final acetic acid concentrations were reduced by 0.5- and 4-fold when controlling *poxB* and *pta* routes, respectively [80].

QS is a cell-to-cell communications phenomenon that automatically regulates target gene expression according to specific signal molecule levels, typically N-acyl homoserine lactones (AHLs) and aryl hydrocarbon receptor-interacting proteins (AIPs) in Gram-negative and Gram-positive bacteria, respectively. According to previous reports, AHL transportation is freely diffusible and accompanied by active efflux, based on differences in length and/or degree of substitution in N-acyl side chains [113,114]. However, AIPs can only penetrate cell membranes using channel proteins. Extracellular AIPs bind and phosphorylate particular receptor proteins (e.g., ComP–ComA, AgrA, and ComD in *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*), which transfer phosphates to a regulatory protein, thus activating QS-associated genes [115,116]. As such, both the intracellular and extracellular concentrations of AHLs and AIPs are the same, resulting in collective regulation effects. Two well-studied QS systems include EsaI-EsaR and LuxI-LuxR from *Pantoea stewartia* and *Vibrio fischeri*, respectively. EsaI and LuxI generate AHLs which combine with the transcriptional factors, EsaR and LuxR, to repress and activate the expression of P_{esaS} and P_{luxI} promoter-controlled genes, respectively. In light of the QS mechanisms, gene expression could be precisely controlled according to the concentration of AHLs or AIPs, which were proportional to the cell density, achieving the goal of balancing cell growth and compound production. In doing so, Dinh and Prather combined these two QS systems into one strain to establish a bifunctional dynamic regulation network (Figure 4(B)) [14]. Using this bifunctional system, compound production was repressed until cell density reached a certain threshold. Once optimized, the synthetic pathway was activated and biomass formation repressed, thus balancing cell growth and biosynthesis. Compared with environment-response dynamic regulation strategies, QS-based extracellular metabolite-response dynamic regulation strategies do not require the artificial control of fermentation parameters.

The transmembrane abilities of some metabolites are decided by their structure and influenced by their protonated states. Thus, intra- and extra-cellular dynamic regulation modes can be controlled by regulating the pH. Recently, Skjoedt et al. observed that *cis*,*cis*-muconic acid was protonated when the pH was lower than 4.5, and it could passively diffuse across the yeast cell membrane [117]. However, when the pH was higher than 6, the un-protonated *cis*,*cis*-muconic acid could not cross the membrane [118]. In this background, using a *cis*,*cis*-muconic acid response TF (BenM), Snoek et al. established a biosensor-selector that automatically selected high titer strains, when the pH was adjusted to 6 [118]. Data from this study revealed that the best producer generated 2 g/L *cis*,*cis*-muconic acid.

A vast number of valuable compounds are synthesized in microbial cell factories. In contrast, few studies have reported on transmembrane transport modes and the spatial distribution state of these products. Thus, while many dynamic regulation strategies have been established for target products, it is difficult to define regulation at spatiotemporal levels [9,119]. In this regard, microdroplet-based fermentation and screening technologies have gained considerable attention [120,121]. A significant advantage of this technology is the separation of individual cells into single microdroplets, thus cellular cross-talk is avoided during fermentation processes. In comparing the sorting effects of single-cell and microdroplet-based fluorescence-activated cell sorting, Wagner et al. observed that secreted products significantly influenced detection signals, and residual intracellular products did not represent the real production states of individual cells [122]. Hence, extracellular production suffered different degrees of the collective regulation problem which depended on the

secrete ability and the solution might be using microdroplet encapsulation technique at present.

Conclusions and prospects

Overall, metabolic heterogeneity and fermentation variability generates considerable challenges in fine-tuning the metabolic flux of individual cells according to intra- and extra-cellular states. To overcome these challenges, environment-response and extracellular metabolite-response dynamic regulation strategies have been established, and collectively regulate the metabolic flux of all cells according to changes in extracellular conditions. On the contrary, intracellular metabolite-response dynamic regulation strategies have specifically regulated the metabolic flux of individual cells, based on changes in intracellular conditions. These dynamic regulatory mechanisms require sophisticated biosensors to dynamically fine-tune metabolic flux for biomass formation and/or compound overproduction. To this end, many intracellular biosensors, with superior performance, have been constructed in recent decades [11,30,42,123]. However, while dynamic range, sensitivity, and specificity are significantly enhanced, biosensors still suffer from long response times caused by transcription and translation issues. Low latency biosensors will help to overcome the delay of signal transition [124], promoting the advancements of instantaneous metabolic regulation, quick disease diagnosis, and accurate high-throughput screening. Possible approaches to obtain low latency biosensors include: developing RNA-based biosensors, fast translation TF-based biosensors, or nano-material-based biosensors. Hence, we believe that quick-response intracellular biosensors will be the next major research focus.

For metabolic engineering applications of engineered intracellular biosensors, lots of dynamic regulatory networks have been established [21]. Their function is to redirect metabolic flux to compound biosynthesis pathways, to avoid negative influences on cell growth. In practice, complicated fermentation processes and genetic backgrounds require multi-layered dynamic regulation circuits to construct robust metabolic networks. These simultaneously and automatically fine-tune dozens of target genes, in different manners (repress or enhance), under any conditions. These strategies which establish and optimize multi-layered dynamic regulation circuits are trial-and-error approaches, using different strength promoters to fine-tune metabolic flux distribution nodes [14,80,125–128]. Such approaches are laborious and often generate sub-optimum results. In this regard, more rational and high-throughput design approaches, such as machine and deep learning, are required in the future. On the other hand, spatiotemporal properties of dynamic regulation are closely related to the distribution of both environment and metabolite inducers. However, transmembrane transport modes of inducers are seldom reported, and thus blur the boundaries of single-cell and collective regulation approaches. Thus, we believe that future research should involve the remodeling of metabolite transportation modes to derive desired spatial distribution properties.

Author contributions

S.Z., Y.D., and J.Z. designed the review. H.S.A. and Y.D. revised the manuscript. S.Z. wrote the manuscript. All authors read and approved the final manuscript.

Disclosure statement

The authors report no declarations of interest.

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