Genetically Encoded Biosensors and Their Applications in the Development of Microbial Cell Factories

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Abstract

The genetically encoded biosensors, which could transform the input of specific metabolic concentrations into output of gene expression levels, have been developed by hacking the sensing and regulatory systems of the cell such as allosteric transcription factors (aTFs) and riboswitches. In this chapter, we first introduce the classification and functional mechanism of genetically encoded biosensor. Furthermore, the applications of biosensor in the development of microbial cell factories including high-throughput screening and dynamic metabolic engineering are reviewed. Finally, the future perspectives on biosensors and their applications are discussed.

Keywords

Biosensor · Allosteric transcription factors · Riboswitch · Synthetic biology · Microbial cell factory · High-throughput screening · Dynamic metabolic engineering

More and more microbial cell factories have been constructed for the production of valuable products such as biofuels, chemicals, materials, and nutraceuticals using renewable biomass sources (Cordova and Alper 2016; Liu et al. 2017a, b; Luo et al.

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2019; Zhou et al. 2018), and this process has been further facilitated by the development of synthetic biology (Ng et al. 2015). Microorganisms have the ability to sense the change of a wide range of metabolites and modulate related pathways accordingly. This process is achieved by their sensing and regulatory systems such as allosteric transcription factors (aTFs) and riboswitches. With the aid of synthetic biology, the genetically encoded biosensors, which were designed and built by engineering the native sensing and regulatory systems of cells, have been widely applied in the high-throughput screening and metabolic regulation of the microbial strains (Koch et al. 2019; Michener et al. 2012). In this chapter, we focused on the constructions and applications of biosensors derived from allosteric transcription factors (aTFs) and riboswitches, and divided them into two categories, namely the protein-based biosensors and the RNA-based biosensors. Other types of biosensors, including the Förster resonance energy transfer (FRET)-based and two-component regulatory system (TCRS)-based biosensors that have not been used widespread in the development of microbial cell factories, will not be discussed here (refer to reviews (Greenwald et al. 2018; Ravikumar et al. 2017)).

4.1 The Classification of Genetically Encoded Biosensors

4.1.1 Protein-Based Biosensors

4.1.1.1 The Functional Mechanism of Protein-Based Biosensors

The protein-based biosensors were usually constructed by engineering aTFs, which could interact with specific small ligand molecules and change the activity of corresponding promoters (Fig. 4.1a) (Table 4.1) (De Paepe et al. 2017). The aTF typically consists of two function domains, namely the N-terminal ligand-binding domain (LBD) and the C-terminal DNA-binding domain (DBD). The binding of

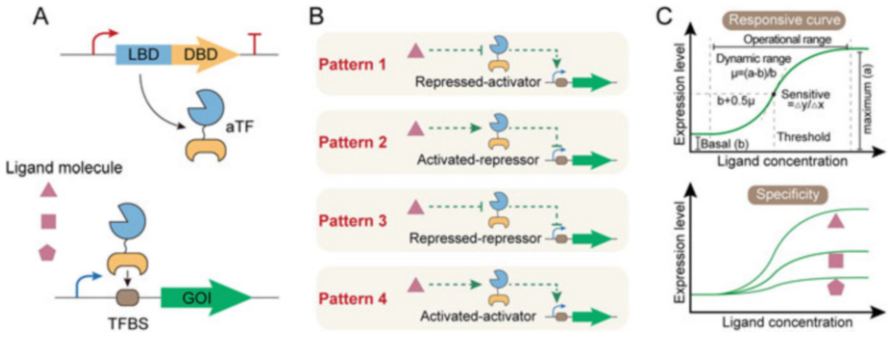

Fig. 4.1 The functional mechanism and fundamental characteristics of protein-based biosensors. (**a**) The two function domains of protein-based biosensors. (**b**) The four general patterns of the aTF-mediated transcriptional regulation. (**c**) The fundamental characteristics of protein-based biosensors

Table 4.1 List of the protein-based biosensors

Ligand	Origin	Host	Application	Refs.
L-Arginine	ArgP (*E. coli*)	*E. coli*	N/a	Binder et al. (2012)
L-Tyrosine	TyrR (*E. coli*)	*E. coli*	Dynamic metabolic engineering (DME)	Chou and Keasling (2013)
L-Phenylalanine	TyrR (*E. coli*)	*E. coli*	High-throughput screening (HTS)	Liu et al. (2017a, b)
FA/acyl-CoA	FadR (*E. coli*)	*E. coli*	DME	Zhang et al. (2012)
Malonyl-CoA	FapR (*B. subtilis*)	*E. coli*	DME	Xu et al. (2014)
Acyl-CoA	FadR (*E. coli*)	*E. coli*	HTS	Xiao et al. (2016)
Adipate	PcaR (*P. putida*)	*E. coli*	HTS	Dietrich et al. (2013)
Glucarate	CdaR (*E. coli*)	*E. coli*	HTS	Rogers et al. (2015)
Glucarate	CdaR (*E. coli*)	*E. coli*	HTS	Zheng et al. (2018)
Acrylate	AcuR (*Rhodobacter sphaeroides*)	*E. coli*	N/a	Rogers et al. (2015)
Ferulic acid	FerC (*Sphingobium*)	*E. coli*	N/a	Machado and Dixon (2016)
Itaconate	ItcR (*Yersinia pseudotuberculosis*)	*E. coli*	HTS	Hanko et al. (2018)
Myo-inositol	IpsA (*C. glutamicum*)	*E. coli*	DME	Doong et al. (2018)
Muconic acid	CatR (*P. putida*)	*E. coli*	DME	Yang et al. (2018)
Ectoine	AraC (*E. coli*)	*E. coli*	HTS	Chen et al. (2015)
NADPH	SoxR (*E. coli*)	*E. coli*	HTS	Siedler et al. (2014a)
N-acetylneuraminic	NanR (*E. coli*)	*E. coli*	HTS	Peters et al. (2018)
Benzoate/2-hydroxybenzoate	NaHR (*P. putida*)	*E. coli*	HTS	van Sint Fiet et al. (2006)
Benzoate	BenR (*P. putida*)	*E. coli*	HTS	Uchiyama and Watanabe (2008)
3,4-Dihydroxy benzoate	PcAU (*Acinetobacter*)	*E. coli*	HTS	Jha et al. (2014)
Kaempferol	QdoR (*B. subtilis*)	*E. coli*	HTS	Siedler et al. (2014b)

(continued)

Table 4.1 (continued)

Ligand	Origin	Host	Application	Refs.
Quercetin	QdoR (*B. subtilis*)	*E. coli*	N/a	Siedler et al. (2014b)
Naringenin	FdeR (*Herbaspirillum seropedicae*)	*E. coli*	N/a	Siedler et al. (2014b)
Naringenin	TtgR (*P. putida*)	*E. coli*	N/a	Rogers et al. (2015)
Vanillin	QacR (*Staphylococcus aureus*)	*E. coli*	N/a	De Los Santos et al. (2016)
Resveratrol	TtgR (*P. putida*)	*E. coli*	HTS	Xiong et al. (2017)
p-Coumaric acid	PadR (*B. subtilis*)	*E. coli*	HTS	Siedler et al. (2017)
Benzoate	Chimeric aTF	*E. coli*	N/a	Juárez et al. (2018)
Pinocembrin/naringenin	FdeR (*Herbaspirillum seropedicae*)	*E. coli*	N/a	Trabelsi et al. (2018)
Vanillin/syringaldehyde	EmrR (*E. coli*)	*E. coli*	HTS	Ho et al. (2018)
Naringenin/apigenin/luteolin	FdeR (*Herbaspirillum seropedicae*)	*E. coli*	HTS	De Paepe et al. (2019)
3-Dehydroshikimate	CusR (*E. coli*)	*E. coli*	HTS	Li et al. (2019)
Arabinose	AraC (*E. coli*)	*E. coli*	N/a	Rogers et al. (2015)
Fucose/gentiobiose/lactitol/ sucralose	LacI (*E. coli*)	*E. coli*	N/a	Taylor et al. (2015)
Cellobiose	CelR (*Thermomonospora fusca*)	*E. coli*	N/a	Kwon et al. (2018)
1-Butanol	BmoR (*Pseudomonas butanovora*)	*E. coli*	HTS	Dietrich et al. (2013)
Phenol	DmpR (*Pseudomonas*)	*E. coli*	HTS	Choi et al. (2014)
3-Hydroxypropionate	PrpR (*E. coli*)	*E. coli*	HTS	Rogers and Church (2016)
3-Hydroxypropionate	AcuR (*R. sphaeroides*)	*E. coli*	HTS	Rogers and Church (2016)
Lactam	ChnR (*Acinetobacter*)	*E. coli*	N/a	Zhang et al. (2017)

Formaldehyde	FrmR (*E. coli*)	*E. coli*	HTS	Woolston et al. (2018)
Choline	BetI (*E. coli*)	*E. coli*	DME	Saeki et al. (2016)
Ammonium	GlnR (*Lactococcus*)	*E. coli*/ *P. putida*/ *synechocystis*	DME	Xiao et al. (2017)
Putrescine	PuuR (*E. coli*)	*E. coli*	HTS	Chen et al. (2017)
Anhydrotetracycline	TetR (*E. coli*)	*E. coli*	N/a	Rogers et al. (2015)
Erythromycin	MphR (*E. coli*)	*E. coli*	N/a	Rogers et al. (2015)
Erythromycin	MphR (*E. coli*)	*E. coli*	HTS	Kasey et al. (2018)
Copper	MarR (*E. coli*)	*E. coli*	N/a	Hao et al. (2014)
Zinc	ZntR (*E. coli*)	*E. coli*	N/a	Watstein et al. (2015)
Arsenite	ArsR (*E. coli*)	*E. coli*	N/a	Merulla and Van Der Meer (2016)
L-lysine/L-arginine/L-histidine	LysG (*C. glutamicum*)	*C. glutamicum*	HTS	Binder et al. (2012)
L-leucine/L-isoleucine/L-methionine/L-valine	Lrp (*C. glutamicum*)	*C. glutamicum*	HTS	Mustafi et al. (2012)
L-lysine/L-arginine/L-histidine	LysG (*C. glutamicum*)	*C. glutamicum*	HTS	Schendzielorz et al. (2014)
Shikimic acid	ShiR (*C. glutamicum*)	*C. glutamicum*	HTS	Liu et al. (2018)
Malonyl-CoA	FapR (*B. subtilis*)	*S. cerevisiae*	DME	David et al. (2016)
Malonyl-CoA	FapR (*B. subtilis*)	*S. cerevisiae*	N/a	Dabirian et al. (2019b)
Acyl-CoA	FadR (*E. coli*)	*S. cerevisiae*	HTS	Dabirian et al. (2019a)
Cis, cis-muconic acid	BenM (*Acinetobacter*)	*S. cerevisiae*	HTS	Skjoedt et al. (2016)
Muconic acid	ARO9 (*S. cerevisiae*)	*S. cerevisiae*	HTS	Leavitt et al. (2017)
S-adenosylmethionine	MetJ (*E. coli*)	*S. cerevisiae*	HTS	Umeyama et al. (2013)
NADH	GPD2 (*S. cerevisiae*)	*S. cerevisiae*	N/a	Knudsen et al. (2014)
Xylose	xylR (*B. xylosus*)	*S. cerevisiae*	HTS	Wang et al. (2016)
3-Hydroxypropionic acid	LysR (*P. denitrificans*)	*P. denitrificans*	N/a	Zhou et al. (2015)
Pamamycin	PamR2 (*Streptomyces alboniger*)	*S. alboniger*	HTS	Rebets et al. (2018)

aTF on the transcription factor binding site (TFBS) of the promoter will increase or decrease the affinity of RNA polymerase (RNAP) to it, and the conformation changes of aTF induced by specific ligand will affect its binding to the promoter thus building a relationship between ligand concentration and promoter activity (Wan et al. 2019). Among the four general patterns of the aTF-mediated transcriptional regulation, patterns 3 and 4 were most employed due to the positive correlation between the input and output (Fig. 4.1b) (Mannan et al. 2017).

The two fundamental characteristics, namely responsive curve and specificity, were often used for the evaluation of the protein-based biosensor (Fig. 4.1c) (De Paepe et al. 2017). The responsive curve represents the relation between the input of ligand concentration and the output of promoter strength, which can be obtained by fitting the input and output into the Hill function as shown below:

$$y = y_{\min} + (y_{\max} - y_{\min}) \frac{x^n}{K^n + x^n} \tag{4.1}$$

where y is relative expression activity of the promoter ($y_{\min}$ and $y_{\max}$ are the minimum/maximum activities), x is the ligand concentration, K is the threshold, and n is the cooperativity (Meyer et al. 2019). And many important parameters of the biosensor could be acquired from the curve including basal, maximum, operational range, dynamic range, threshold, and sensitivity (Fig. 4.1c). Specificity determines the responsive of the biosensor to different ligand molecules.

4.1.1.2 Designing and Tuning Protein-Based Biosensors

In order to build a protein-based biosensor with favorable responsive curve in a host, specific aTF should be expressed properly, and applicable synthetic promoter needs to be designed and constructed. Sometimes, molecular modification on the aTF may be implemented to improve or change the specificity of biosensor (De Paepe et al. 2017). That is to say, the tuning of protein-based biosensor mainly focuses on aTF level and promoter level.

Tuning at aTF Level

To construct a protein-based biosensor responsive to a specific molecule, corresponding aTF must be chosen by consulting literatures or retrieving the databases such as RegulonDB (Gama-Castro et al. 2011), BRENDA (Placzek et al. 2017), and RegPrecise (Rodionov et al. 2013). Besides, transcriptome sequencing and analysis can also be used to identify specific aTF (Li et al. 2019). However, there may not be aTF in nature which responds to certain molecules. So the engineered aTFs responded to new non-natural ligands must be constructed, which could be achieved by the combination of rational design and directed evolution (Koch et al. 2019; Libis et al. 2016). For example, five amino acid positions located in the effector binding pocket (P8, T24, H80, Y82, and H93) of the L-arabinose-responsive aTF AraC were selected for simultaneous saturation mutagenesis, and the mutants that responded to mevalonate, triacetic acid lactone, and ectoine, respectively, were obtained by fluorescence-activated cell sorting (FACS)-mediated

negative–positive dual screening (Chen et al. 2015; Tang et al. 2013; Tang and Cirino 2011). The computational design method is often used to reduce the design space. As an example, the Rosetta software was used in combination with single-residue saturation mutagenesis and error-prone PCR (epPCR)-based random mutagenesis for the construction of LacI mutants responding to fucose, gentiobiose, lactitol, and sucralose, respectively (Taylor et al. 2016). In addition, chimeric aTFs have also been built by fusing DBD and LBD from different proteins, and it is worth mentioning that the LBD could come from proteins other than aTF as long as it has demonstrable binding affinity to the ligand. For instance, benzoate-responsive aTFs were constructed by connecting benzoate LBDs to different DBDs with optimized linkers (Juárez et al. 2018).

The fundamental characteristics of the protein-based biosensors can also be optimized by introducing molecular modification into or tuning the expression level of the aTF. For example, the specificity of aTFMphR (that is derepressed by several naturally produced and semisynthetic macrolide antibiotics including erythromycin (ErA), josamycin, oleandomycin, narbomycin, methymycin, and pikromycin) to erythromycin was enhanced through epPCR and FACS; and its sensitivity was improved by introducing random mutagenesis to ribosome binding site (RBS) fortuning its expression level (Kasey et al. 2018).

Tuning at Promoter Level

To build a protein-based biosensor in a host, synthetic responsive promoters need to be designed and constructed by inserting the TFBS into the promoter of this strain because the native promoter regulated by the aTF may lose its activity there. For example, FA/acyl-CoA-responsive promoters were built by inserting the TFBS of aTF FadR into a phage lambda promoter and a phage T7 promoter, respectively, and TFBS of LacI was added into the constructed synthetic promoters to eliminate leaky expression (Zhang et al. 2012). In addition, the fundamental characteristics could be modulated by changing the starting engineered promoter or the position and numbers of the TFBS. As an example, Siewers and coworkers have constructed several malonyl-CoA biosensors in *Saccharomyces cerevisiae* by inserting the TFBS of aTF FapR (FapO) into five native promoters, and improved the dynamic range and reduced the basal by adjusting the position and numbers of FapO (Dabirian et al. 2019b).

4.1.2 RNA-Based Biosensors

4.1.2.1 The Functional Mechanism of RNA-Based Biosensors

The RNA-based biosensors could be constructed by engineering the cis-acting metabolite-responsive riboswitches, which consist of ligand-binding (aptamer) domains that could bind with specific ligand when its abundance exceeds a threshold and expression platform that control the gene expression by interacting with various gene expression apparatus (Table 4.2) (Serganov and Patel 2007). In the natural world, riboswitches responsive to numerous small molecules including ion, purines,

Table 4.2 List of the RNA-based biosensors

Ligand	Origin	Host	Application	Refs
L-lysine	Lysine riboswitch (*E. coli*)	*E. coli*	HTS	Yang et al. (2013)
L-lysine	Lysine riboswitch (*E. coli*)	*E. coli*	HTS	Wang et al. (2015)
L-tryptophan	L-tryptophan riboswitch (artificial)	*E. coli*	HTS	Yang et al. (2013)
L-tryptophan	L-tryptophan riboswitch (artificial)	*E. coli*	N/a	Jang and Jung (2018)
Thiamine 5′-pyrophosphate (TPP)	TPP riboswitch (*E. coli*)	*E. coli*	N/a	Nomura and Yokobayashi (2007)
TPP	TPP riboswitch (artificial)	*E. coli*	N/a	Wieland et al. (2009)
TPP	TPP riboswitch (*E. coli*)	*E. coli*	N/a	Muranaka et al. (2009)
TPP	TPP riboswitch (*E. coli*)	*E. coli*	N/a	You et al. (2015)
Theophylline	Theophylline riboswitch (artificial)	*E. coli*	N/a	Lynch et al. (2007)
Theophylline	Theophylline riboswitch (artificial)	*E. coli*	N/a	Win and Smolke (2007)
Theophylline	Theophylline riboswitch (artificial)	*E. coli*	N/a	Wieland and Hartig (2008)
Theophylline	Theophylline riboswitch (artificial)	*E. coli*	N/a	Lynch and Gallivan (2009)
Theophylline	Theophylline riboswitch (artificial)	*E. coli*	N/a	Wachsmuth et al. (2013)
Theophylline	Theophylline riboswitch (artificial)	*E. coli*	HTS	Eckdahl et al. (2015)
Theophylline	Theophylline riboswitch (artificial)	*E. coli*	N/a	Page et al. (2018)
Flavin mononucleotide (FMN)	FMN riboswitch (artificial)	*E. coli*	HTS	Meyer et al. (2015)
Flavin mononucleotide (FMN)	FMN riboswitch (*Fusobacterium nucleatum*)	*E. coli*	N/a	Rode et al. (2015)
Vitamin B12	Vitamin B12 riboswitch (*Propionibacterium freudenreichii*)	*E. coli*	N/a	Zhu et al. (2015)
Theophylline/ tetramethylrosamine/fluoride/ dopamine/thyroxine/2,4-dinitrotoluene	Artificial riboswitches based on related aptamer	*E. coli*	N/a	Espah Borujeni et al. (2016)
S-adenosyl-L-homocysteine (SAH)	SAH riboswitch (*Ralstonia solanacearum*)	*E. coli*	HTS	Su et al. (2016)

Neomycin thiamine/TPP/*S*-adenosyl methionine (SAM)/adenine (ade)	Artificial riboswitches based on related aptamer	*E. coli*	N/a	Endoh and Sugimoto (2015)
Purine	Purine riboswitch (*B. subtilis*)	*E. coli*	N/a	Stoddard et al. (2013)
Glycine	Glycine riboswitch (*B. subtilis*)	*E. coli*	N/a	Ketterer et al. (2016)
Ammeline/azacytosine	Adenine riboswitch (*Vibrio vulnificus*)	*E. coli*	N/a	Dixon et al. (2010)
Naringenin	Naringenin riboswitch (artificial)	*E. coli*	N/a	Jang et al. (2017)
Naringenin	Naringenin riboswitch (artificial)	*E. coli*	N/a	Xiu et al. (2017)
N-acetylneuraminate	*N*-acetylneuraminate aptazyme (artificial)	*E. coli*	HTS	Yang et al. (2017)
Neomycin	Neomycin riboswitch (artificial)	*E. coli*	N/a	Weigand et al. (2008)
Atrazine	Atrazine riboswitch (artificial)	*E. coli*	N/a	Sinha et al. (2010)
Pyrimido[4,5-d]pyrimidine-2,4-diamine (PPDA)	PPDA riboswitch (artificial)	*E. coli*	N/a	Kent and Dixon (2019)
5-hydroxytryptophan/3,4-dihydroxyphenylalanine	Artificial riboswitches	*E. coli*	N/a	Porter et al. (2017)
Ni2+/Co2+	NiCo riboswitch (*Clostridium botulinum*)	*E. coli*	HTS	Furukawa et al. (2015)
Glucosamine-6-phosphate (GlcN6P)	GlcN6P riboswitch (*B. subtilis*)	*B. subtilis*	DME	Niu et al. (2018)
L-lysine	L-lysine riboswitches (*E. coli* and *B. subtilis*)	*C. glutamicum*	DME	Zhou and Zeng (2015a)
L-lysine	L-lysine riboswitch (*E. coli*)	*C. glutamicum*	DME	Zhou and Zeng (2015b)
GlcN6P	GlcN6P riboswitch (*B. subtilis*)	*S. cerevisiae*	HTS	Lee and Oh (2015)
Theophylline	Theophylline riboswitch (artificial)	*S. cerevisiae*	N/a	Klauser et al. (2015)
Theophylline	Theophylline riboswitch (artificial)	*E. coli/Acinetobacter baylyi/ Acinetobacter baumannii/ Agrobacterium tumefaciens/ Streptococcus pyogenes/B. subtilis*	N/a	Topp et al. (2010)

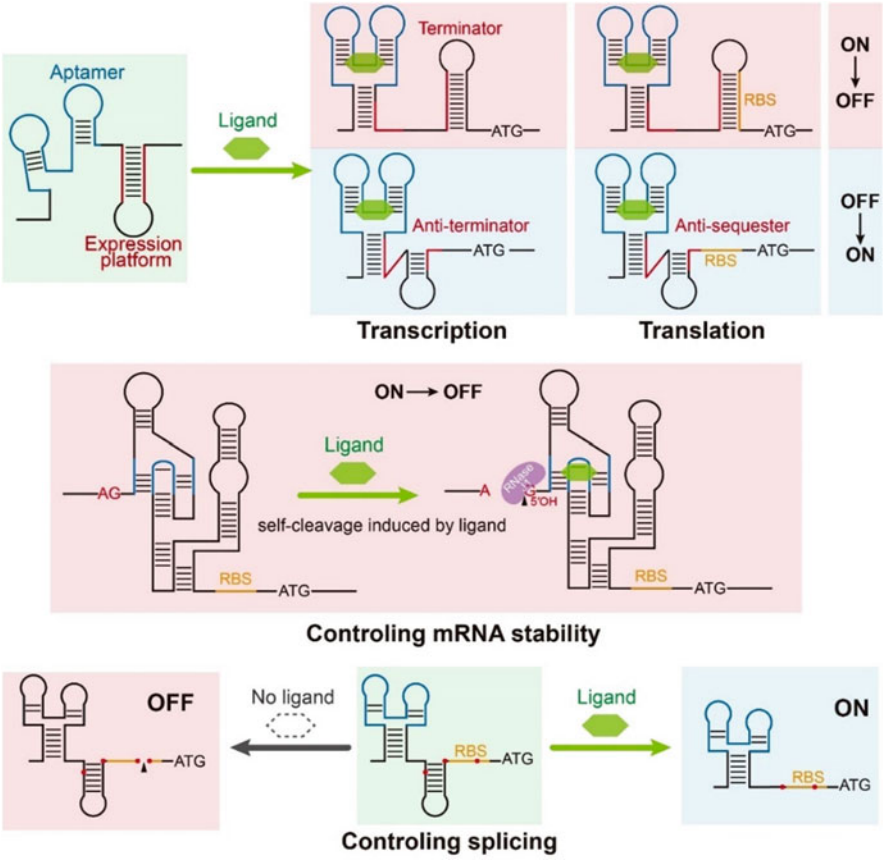

Fig. 4.2 The functional mechanism of riboswitch

and their derivatives, amino acids, phosphorylated sugar, and so on have been found, and they could modulate gene expression by controlling transcription, translation, mRNA stability, and splicing (Fig. 4.2) (Serganov and Nudler 2013). Because the regulations on genes expression are achieved by modulating the secondary structure of mRNAs, RNA-based biosensors possess faster responses compared with the protein-based biosensors. In addition, they have a good transplantable character on account of the protein-free control process (Topp et al. 2010). For example, the glucosamine-6-phosphate riboswitch of *B. subtilis* was directly used for high-throughput screening of *N*-acetylglucosamine high-producing strain in *S. cerevisiae* (Lee and Oh 2015). The RNA-based biosensors also function in a dose-dependent manner, hence their fundamental characteristics for evaluation are the same as the protein-based biosensors mentioned above (Chang et al. 2012).

4.1.2.2 Designing and Tuning RNA-Based Biosensors

Duo to functional mechanism of riboswitches, the RNA-based biosensors are easily to be designed and built by adding the natural or engineered riboswitches into mRNAs (usually on 5′ untranslated region (UTR)), and the responsive characteristics could be tuned by modifying their sequences in aptamer region or expression platform and getting the mutants using high-throughput screening (Jang and Jung 2018; Page et al. 2018).

The dynamic range of the RNA-based biosensors can be improved by changing their promoter or RBS, and the anti-RBS sequence on the riboswitch also needs to be modified if it functions in an RBS sequestering manner. For example, RBS sequence on expression platform of the pyrimido[4,5-d]pyrimidine-2,4-diamine (PPDA) riboswitch was exchanged with the *E. coli* consensus RBS sequence (AGGAGG) for enhanced maximum of the biosensor firstly, and then high-throughput fluorescence-activated cell sorting (FACS)-based selection/counter selection methodology was used to identify anti-RBS sequences that give riboswitches with optimal OFF and ON states. Introducing these modifications improved the maximal expression and dynamic range of the biosensor by 8.2-folds and 80-folds, respectively (Kent and Dixon 2019). As an another example, Jiang et al. improved the dynamic range of a L-tryptophan riboswitch-based biosensor by changing its promoter and copy number (Jang and Jung 2018). To modulate the operational range of the riboswitch-based biosensor, the aptamer region can be modified to change the affinity between ligand and riboswitch. For instance, the dose–response curve of a L-tryptophan riboswitch-based biosensor was shifted toward higher ligand concentrations by exchanging a low affinity aptamer (Jang and Jung 2018).

The ligand specificity of the RNA-based biosensors may be enhanced or changed by modifying the aptamer regions (Robinson et al. 2014). For instance, the specially responsive ligand of the natural adenine riboswitch was turned to ammeline or azacytosine by introducing site-directed mutagenesis at U47 and U51 sites on the aptamer region that are responsible for the interaction with the ligand molecule (Dixon et al. 2010). In addition, "non-natural" synthetic riboswitches could be designed and constructed using corresponding aptamers found in the natural world or built artificially (Darmostuk et al. 2014; Kinghorn et al. 2017; Sun and Zu 2015). For example, an L-tryptophan riboswitch was built by selecting the N_{10} sequences connecting L-tryptophan aptamer region that had been reported previously with RBS and dual selection module (tetA-sgfp) in vivo (Yang et al. 2013). In addition, a statistical thermodynamic model has been proposed for the aptamer-based artificial riboswitch design (Espah Borujeni et al. 2016). In another example, self-cleaving ribozyme-based artificial riboswitches have been built by linking the *thiM* aptamer domain from *E. coli* into stem III of a fast-cleaving hammerhead ribozyme (HHR) (Wieland et al. 2009).

It is worth mentioning that new artificial RNA aptamers that bind to specific ligands could be easily constructed using the technology called systematic evolution of ligands by exponential enrichment (SELEX) in vitro (Darmostuk et al. 2014), and then the new aptamers will be used for the building of corresponding riboswitches (Jang et al. 2017). Alternatively, riboswitches responsive to new ligand can be also

constructed by directly introducing a random-sequence library into the aptamer domain of a native or ready-made riboswitch and then conducting multiple rounds of dual genetic selection and FACS screening in vivo. Using this method, theophylline riboswitch that possesses a 2.3-fold dynamic range was obtained from the native ThiM#2 riboswitch (Page et al. 2018).

4.2 The Application of Genetically Encoded Biosensors

4.2.1 The Application in High-Throughput Screening

Because the metabolic networks and their regulations are very complex in the cell, high-throughput screening (HTS) is often used to obtain the best producer from the mutant libraries of enzymes or pathways (Lim et al. 2018). The genetically encoded biosensors could couple the target products' concentrations with expression levels of the reporters, and then the best producer can be obtained by adaptive evolution or FACS.

4.2.1.1 Screening by Adaptive Evolution

To carry out adaptive evolution, appropriate reporter needs to be chosen to link cell growth with product concentration. For the biosensors whose expression levels are positive correlation to the concentrations of ligands, resistance maker could be used. For example, a tetracycline resistance protein TetA was used as the reporter of the aTF-based biosensor for directed evolution of a heterologous biosynthetic pathway of 1-butanol in *E. coli* (Dietrich et al. 2013). As the biosensors whose expression levels possess negative correlation with the ligand concentrations, negative selection marker needs to be used. For example, cytosine deaminase that has a cytotoxicity was used as the reporter of the GlcN6P riboswitch-based biosensor for the screening of the best mutant of the key pathway enzyme GFA1 for N-acetylglucosamine (GlcNAc) synthesis in *S. cerevisiae* (Lee and Oh 2015).

4.2.1.2 Screening by Fluorescence-Activated Cell Sorting

The genetically encoded biosensors could also be applied for FACS by using fluorescence protein as the reporter. For instance, the yellow fluorescence protein (YFP) was acted as the reporter of a lysine biosensor in *C. glutamicum*, and then FACS was conducted for screening of pyruvate carboxylase variants created by error-prone PCR that enable improved L-lysine production from glucose (Kortmann et al. 2019).

4.2.2 The Application in Dynamic Metabolic Engineering

The genetically encoded biosensors also have wide applications in dynamic metabolic engineering, which is capable of dynamically coordinating the metabolic flux in a feedback manner and can avoid the adverse effects on cells caused by metabolic

modification such as metabolic imbalance and accumulation of intermediate products (Lalwani et al. 2018; Shen et al. 2019; Xu 2018). Here, we divide these applications into three categories according to the regulation processes, namely dynamic pathway activation, dynamic pathway repression, and dynamic dual control (simultaneous activation and repression).

4.2.2.1 Dynamic Pathway Activation

Dynamic pathway activation can be used to redirect the flux from the native metabolism toward the target product by introducing a biosensor responsive to prevalent intermediate at the key branch points in the metabolic networks of the cell. For example, amalonyl-CoA biosensor was employed to alter the metabolic flux from central carbon metabolism into a heterologous 3-hydroxypropionic acid (3-HP) synthetic pathway by controlling the expression of the malonyl-CoA reductase derived from *Chloroflexus aurantiacus*, which enabled the dynamic switching between growth phase and production (David et al. 2016).

4.2.2.2 Dynamic Pathway Repression

The competitive pathways of target product were often knocked-outed to force more metabolic flux into the pathway of interest, while sometimes these competitive pathways may be necessary for the cell growth. In this situation, dynamic repression can be employed to redirect the flux toward target product. For instance, the lysine-OFF riboswitch was used to control the expression of citrate synthase (*gltA*), which is the key metabolic point of tricarboxylic acid (TCA) cycle, in a L-lysine-producing *C. glutamicum* strain, thus dynamically channel flux from central carbon metabolism into L-lysine synthesis (Zhou and Zeng 2015a). Similarly, a GlcN6P-OFF riboswitch was set as an intermediate metabolite biosensor that dynamically repressed the competitive pathways, namely peptidoglycan synthesis pathway and glycolysis pathway, in a GlcNAc-producing *B. subtilis* strain (Niu et al. 2018).

4.2.2.3 Dynamic Dual Control

To achieve the better and more precise control of the metabolic networks in a microbial cell factory, dynamic activation and repression on multiple targets simultaneously, which is widespread in the natural world, may be needed. This process can be realized by designing and building biosensors that possess opposite regulation effects. Xu et al. have constructed malonyl-CoA activating and repressing biosensors regulated by the aTF FapR, and controlled the malonyl-CoA source pathway (ACC) and the malonyl-CoA sink pathway (FAS) by the malonyl-CoA activating and repressing biosensors, respectively, which avoided the accumulation of intermediate product malonyl-CoA and balanced metabolism between cell growth and target product fatty acids formation (Xu et al. 2014). In another example, lysine-ON riboswitches were built by engineering a native lysine-OFF riboswitch from *E. coli*, and lysine-ON and lysine-OFF riboswitches were applied for the control of lysine transport protein and the key competitive pathway, namely TCA cycle, respectively (Zhou and Zeng 2015a).

Except for the double sensor mediated dynamically dual control, single biosensor-based dual control, which could be achieved by coupling the biosensor with some regulation tools acted as NOT gates, have also been reported. Yan and coworkers have presented a bifunctional dynamic control system based on biosensor and antisense RNA (as RNA), which can be used to upregulate and downregulate multiple genes simultaneously, and applied this system to achieve the dynamic flux distribution between native metabolism and the muconic acid biosynthetic pathway (Yang et al. 2018). In addition, the CRISPRi based NOT gate was also coupled with a biosensor to achieve the autonomous dual-control of metabolic flux in *Bacillus subtilis* (Wu et al. 2020).

4.3 Conclusions and Perspectives

Genetically encoded biosensors have been widely applied in the construction of efficient microbial cell factories. However, the building process of novel biosensors responsive to specific macular, which is the premise of all subsequent operations, is still time-consuming. Hence the computer-aided methods need to be further explored for accelerating biosensor design in the future. In addition, the biosensors-mediated feedback and dynamic regulation of the metabolic networks can be combined with the rising co-culture engineering strategy, which has been proved to be more advantageous in the synthesis of many products (Jones and Wang 2018), to achieve the coordination control of population dynamics. Furthermore, biosensors may also be used in the regulation of engineering spatial organization of metabolic enzymes, which can enhance flux into interested pathway and reduce their interactions with cellular background metabolism (Lee et al. 2012), for the reconstruction of the cell metabolism in space and time dimensions simultaneously.

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