

Review

Genetic Biosensor Design for Natural Product Biosynthesis in Microorganisms

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Low yield and low titer of natural products are common issues in natural product biosynthesis through microbial cell factories. One effective way to resolve such bottlenecks is to design genetic biosensors to monitor and regulate the biosynthesis of target natural products. In this review, we evaluate the most recent advances in the design of genetic biosensors for natural product biosynthesis in microorganisms. In particular, we examine strategies for selection of genetic parts and construction principles for the design and evaluation of genetic biosensors. We also review the latest applications of transcription factor- and riboswitch-based genetic biosensors in natural product biosynthesis. Lastly, we discuss challenges and solutions in designing genetic biosensors for the biosynthesis of natural products in microorganisms.

Necessity of Genetic Biosensors for Biosynthesis of Natural Products

Much progress has been made recently in engineering microorganisms for the biosynthesis of natural products, such as secondary metabolites, antibiotics, and fatty acids [1–4]. However, efficient biosynthesis via microbial systems is still limited, due to issues such as low **productivity** (see [Glossary](#)), low **titer**, and low **yield** [5]. One key reason is that engineering of biosynthetic pathways in microbial systems can cause metabolic imbalance between cell growth and the production of target products [6]. Therefore, it is essential to maintain a balance of cellular resources and maximize the metabolic flux towards the target products. Moreover, the metabolites produced are mainly measured using analytical methods such as gas and liquid chromatography, which is time-consuming and relatively low in throughput [7]. For instance, LC-MS or GC-MS can make only up to a threshold of 100–1000 measurements of metabolites per instrument per day [8]. Such bottlenecks impede the process of microbial engineering for producing natural products at a high level.

Designing genetic **biosensors** can expedite the process for optimization of **microbial cell factory** development. First, biological architecture and genetic regulatory parts [e.g., promoter, transcriptional regulator, 5' and 3' untranslated region (UTR) of mRNA] have been designed, constructed, and optimized to regulate and balance biosynthetic pathways for improving the production of natural products [9–11]. Furthermore, genetic biosensor design has been integrated with high-throughput screening (HTS) approaches to accelerate the process for the selection of **genetic parts**, metabolic pathways, and producer strains [10,12–15]. Hence, genetic biosensors have the potential to resolve key bottlenecks in natural product biosynthesis in microbial hosts.

In this review, we evaluate the most up-to-date advances in the design of genetic biosensors for natural product biosynthesis in microorganisms. We extend and expand prior reviews on small molecule biosensors, transcriptional biosensors, yeast biosensors, and cell-based biosensors [16–21]. Specifically, we examine the most recent strategies for the selection of genetic parts,

Highlights

The implementation of genetic biosensors offers opportunities to resolve key bottlenecks in natural product biosynthesis.

Genetic biosensors are powerful tools for real-time monitoring of natural product biosynthesis in microbial cell factories.

Coupled design of genetic biosensors with high-throughput analytics in biofoundries can accelerate the process for selecting genetic parts, metabolic pathways, and producer strains.

Combining transcription factors and riboswitches to complement one another's functions has emerged to improve natural product biosynthesis.

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and construction principles for the design and evaluation of genetic biosensors. We also review the most recent applications of **transcription factor** (TF)- and **riboswitch**-based genetic biosensors which can either be used for HTS of product formation or for fine-tuning genes in metabolic pathways to enhance product formation. Lastly, we discuss challenges in designing genetic biosensors and technological platforms, such as HTS and biofoundries, that could offer solutions to the challenges.

Genetic Biosensor Design

Strategies for Selection of Genetic Parts for Genetic Biosensors

A genetic biosensor is made up of a sensing device and a transduction device formed by genetic parts [22]. The sensing device serves to detect the existence of an input signal such as metabolite and ligand. It contains a TF (transcriptional activator, transcriptional repressor) consisting of a DNA-binding domain (DBD) and a ligand-binding domain (LBD), or a riboswitch comprising an RNA **aptamer**. The transduction device translates the input signal into an output signal (e.g., fluorescence, colorimetry, and resistance). It contains a reporter gene or pathway genes. The sensing device is functionally linked to the transduction device through the binding of the input signal to a TF or a riboswitch, activating or repressing transcription or translation of genes of interest. In TF-based biosensors, mediated by DBD and/or LBD, transcriptional activators activate transcription of reporter genes by binding to promoters, and transcriptional repressors repress transcription of actuator genes by dissociating from promoters or binding to a corepressing ligand in an allosteric manner [23] (Figure 1A). In the case of chimeric TF-based biosensors, DBD and LBD are linked by a linker to allow flexibility for binding to DNA and ligand [24]. In the case of dimeric or oligomeric TF-based biosensors, both ligand specificity and the correlation between input signal and output signal can be determined [25]. Properties of TFs, such as binding specificity and transduction capability, are associated to the operational range of biosensors, which are important elements for rational design of TF-based biosensors. In a riboswitch-based biosensor, mediated by an RNA aptamer domain, the riboswitch can regulate the processes of transcription via inhibition of antiterminator, post-transcription via cleavage of mRNA, or translation via sequestration of ribosome binding sites (RBSs) [26]. The regulation is realized mainly based on a conformational change through direct binding to a ligand or metabolite (Figure 1B).

Functions of genetic biosensors are determined by genetic parts. Hence, selection of genetic parts is critical in genetic biosensor design. Several approaches are used for the selection of genetic parts (Figure 1C). First, transcriptome and proteome analyses identify TFs and the promoters that they bind to through gene expression and protein profiling in the presence of an input signal. This approach is exemplified by identification of TFs for the detection of 1-butanol [27], FPP [28], and threonine [29]; however, the approach is limited when the transcriptional regulation is unspecific or indirect. Second, chemoinformatics approaches enable potential TF-promoter pairs to be identified [30]. Through chemical similarity search, this approach identifies catabolizable chemicals with molecular shapes similar to metabolic engineering targets, and explores catabolizing gene clusters of such catabolizable chemicals to find inducible TF candidates. Third, systematic evolution of ligands by exponential enrichment (SELEX) generates artificial riboswitches through systematic evolution of ligands [7,31,32]. Specifically, a library of random nucleotide target-binding sequences with an invariable flanking sequence at each terminal are synthesized, selected and enriched. Successful use of other approaches, such as computer-aided mining of literature and databases (Box 1), rational design, and random mutagenesis (Figure 1C) has also been demonstrated. For example, TFs have been selected by mining databases (<http://sensipath.micalis.fr>) and the literature, and using predictive tools (<http://regulondb.ccg.unam.mx>) [8,33,34]. Analysis of conserved DBDs in major TF families has guided selection and rational design of TFs [35]. Compared with other genetic parts, selection of reporters

Glossary

Aptamer: short oligonucleotides (with a length of 30–80 nucleobases) that binds to a specific target molecule.

Biosensor: an analytical device that incorporates a biological system intimately associated with or integrated within a physicochemical detector, can be used to detect chemical substances.

Genetic parts: genetic units that carry out a specific function within a system or a device.

Microbial cell factory: microbial cells that serve as a production facility for metabolites or proteins.

Productivity: mass of a product per culture volume and time, expressed as g/l/h.

Riboswitch: regulatory element in the UTR of mRNA molecules, binds to a small molecule, and changes its conformation that affect the translation of its target protein.

Titer: mass of a product per culture volume, expressed as g/l.

Transcription factor: sequence-specific DNA-binding protein that regulates transcription from DNA to messenger RNA by binding to a specific DNA sequence.

Yield: mass of a product per feedstock mass or final biomass, generally expressed as g/g carbon source, or g/g dry cell weight.

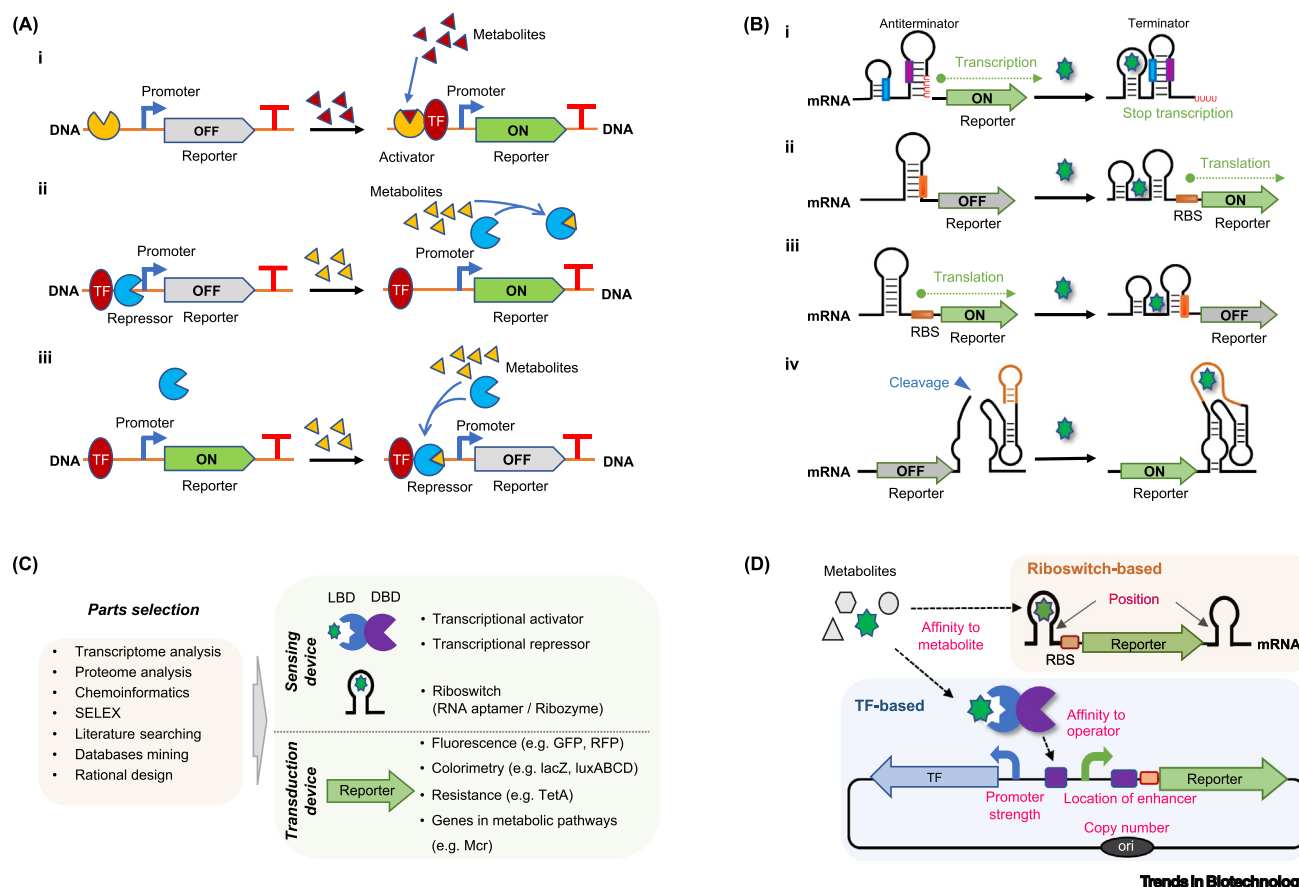


Figure 1. Genetic Parts and Construction for Genetic Biosensor Design. (A) Transcriptional regulation by transcription factor (TF). Allosteric TF binds with a metabolite (i) to form an active transcriptional recruiter (transcriptional activator), for example, LysR-type transcriptional regulators [104]; (ii) to remove a roadblock by releasing from promoter (transcriptional repressor); or (iii) to form an active repressor as a roadblock (transcriptional repressor) to regulate transcription of an actuator gene. (B) Regulation by riboswitch. The riboswitch binds with a metabolite (i) to inhibit antiterminator that breaks formation of a terminator hairpin, leading to stopped transcription of the reporter gene; (ii) to activate or (iii) repress translation of a reporter gene via its conformational change; or (iv) to stabilize mRNA by blocking cleavage site in mRNA which leads to active translation of a reporter gene. (C) Selection of genetic parts. A sensing device consists of genetic parts, for example, transcriptional activator and repressor, and riboswitch. A transduction device consists of genetic parts including genes that encode fluorescent proteins, colorimetric enzymes, antibiotic resistant enzymes, and genes that are regulated in a dynamic manner to enhance metabolic fluxes towards product formation. (D) Principles for construction of genetic biosensors. Several factors indicated should be considered during the construction. Abbreviations: DBD, DNA-binding domain; LBD, ligand-binding domain; SELEX, systematic evolution of ligands by exponential enrichment.

(e.g., GFP, RFP, lacZ, luxABCD, TetA, and Mcr) [36] is straightforward. The selected reporter is usually used for HTS of other genetic parts such as promoters, TFs, and riboswitches, and for screening of optimal natural products biosynthesis pathways from large libraries of design variants (Figure 1C).

Construction Principles for Design and Evaluation of Genetic Biosensors

Following the selection of genetic parts for genetic biosensors, the selected genetic parts are assembled to construct genetic biosensors that can give different modes of output. Constructing genetic biosensors is dependent on the binding affinity of metabolites to TFs or riboswitches, and TFs to operators, as well as promoter strength, plasmid copy number, and the position of functional domains in the mRNA (Figure 1D). First, modular synthetic promoters are built to construct logic gates in order to incorporate dual input signals into one genetic biosensor [37,38]. Mazumder and McMillen constructed a dual-mode promoter that consists of five steroid

Box 1. Databases for Genetic Parts and Regulatory Networks**Prokaryotic TFs**

RegulonDB (<http://regulondb.ccg.unam.mx>): By using this database, it is possible to predict whether the regulators in *E. coli* bind the heterologous promoter integrated from external gene sources or not [99].

Sigmol (<http://bioinfo.imtech.res.in/manojk/sigmol/>): This is a repertoire of the quorum sensing signaling molecules in prokaryotes [100].

SensiPath (<http://sensipath.micalis.fr>): This tool supports genetic parts to connect target compounds to the information in several transcriptional regulation databases and helps to search the data related to detectable compounds on RegPrecise, RegTransbase, BioNemo, or RegulonDB [34].

Eukaryotic TFs

TRANSFAC (<http://gene-regulation.com/pub/databases.html>): It provides the data of eukaryotic transcription factors, e.g., experimentally proven binding sites, consensus binding sequences (positional weight matrices) and regulated genes.

YEASTRACT (<http://www.yeasttract.com/>): It provides a curated repository of approximately 200 000 regulatory associations for correlation between TFs and target genes in *S. cerevisiae*.

YeTFaSCo (<http://yetfasco.cabr.utoronto.ca/>): It is an extensive collection of *S. cerevisiae* TF specificities by evaluating the motifs using multiple objective quality metrics.

Riboswitches

RiboD (<http://ribod.iiserkol.ac.in/>): It compiles prokaryotic riboswitches for 1777 prokaryotic genomes and covers 31 metabolite and ion sensing riboswitch classes [101].

Rfam (<http://rfam.xfam.org/>): This is a database of RNA families, and each RNA family is represented by a multiple sequence alignment, a consensus secondary structure, and a covariance model [102].

RiboGap (<http://ribogap.iaf.inrs.ca/>): It is a database that helps to find intergenic regions (IGRs) from the 5' and 3' UTRs for one or several genes, and provides information on the presence of known noncoding RNAs and Rho-independent transcription terminators in the IGRs [103].

Regulatory Network

Yeast Combinatorial Regulation Database (<http://cosbi.ee.ncku.edu.tw/YCRD/>): It provides associations between cooperative TF pairs and genes of interest.

Saccharomyces Genome Database (<https://www.yeastgenome.org/>): It provides comprehensive integrated information of *S. cerevisiae* with search and analytic tools to explore these data, enabling the discovery of functional relationships between sequence and gene products in fungi and higher organisms.

hormone responsive elements upstream and one lac operator downstream of the TATA box in a minimal cytochrome C promoter. This dual controller allows activation by testosterone and repression by IPTG [37]. Teo and Chang constructed an AND-gate dynamic controller by fusing inducible promoter enhancer sequences with a modular synthetic GAL1 core promoter containing a fatty acyl-CoA operator. The AND-gate controller turns on GFP expression when two inputs (fatty acid and copper presence/phosphate starvation) are present [38]. Second, performance of genetic biosensors is associated with the copy number of the plasmid used. It is essential to use a plasmid with an appropriate origin of replication to construct genetic biosensors. For instance, a low copy number replication origin can tune down the expression. Third, the capability of TFs to control gene expression by detecting a wide range of natural metabolic intermediates makes them suited for constructing genetic biosensors based on transcriptional regulations. In the TF-based biosensors, metabolites serve as input signals to bind with TFs, and the metabolite–TF complex binds onto operators/promoters and controls the expression of target proteins [39,40]. Fourth, constructing riboswitch-based genetic biosensors is based on the structure of riboswitches carrying a target-binding nucleotide sequence selected via SELEX. In a riboswitch, there are two functional domains, LBD and effector domain, located at the 5' or 3' UTR of mRNA,

and physically connected to make an RNA switch, which can affect the binding of ribosomes to the mRNA of a reporter gene, as well as the termination of polymerase reaction by forming a terminator. Hence, rearrangement of the structural configuration between the LBD and the effector domain can achieve sensing in different control modes. As a flexible platform, RNA devices can be combined with another RNA-based architecture for expanding their functions, exemplified by the combination of the hammer head ribozyme and theophylline aptamer [41], and the combination of the twister ribozyme, theophylline aptamer, and thiamine pyrophosphate (TPP) aptamer [42] (Figure 2A). Also, riboswitch engineering can be used to construct novel genetic biosensors. For example, a natural riboswitch that represses the expression of the downstream gene in response to TPP was engineered to activate the expression instead in response to TPP in *Escherichia coli* [43]. Lastly, strand displacement reaction (SDR) based on physicochemical stability of DNA can be used to detect natural products such as biotin, vitamin D, and folate at nanomolar levels by using an SDR-based biosensor. This type of genetic biosensor has shown high sensitivity, low interference, and high controllability [44,45].

The constructed genetic biosensors are evaluated based on the operational range of the biosensor, that is, the range of the concentration of the target metabolite (input signal) required for the biosensor to provide a significant change in output signal. The input signal enables the HTS of microbial cells with diverse pathway designs to produce target natural products within a fixed operational range of the biosensor even after altering the expression level of the metabolic pathway genes [11]. It is affected by the expression level of the reporter, or by signal amplification by using an enzymatic reporter [11]. The dynamic range of the output signal can be expanded by

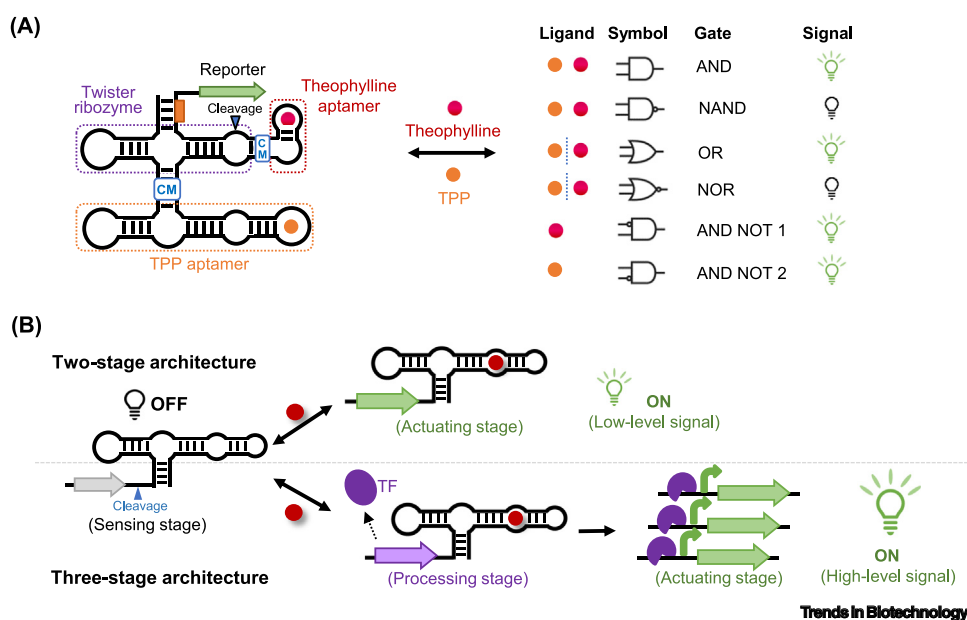


Figure 2. Schematic Presentation of Biosensing Devices for Genetic Biosensors. (A) Hybrid riboswitch controller. The hybrid riboswitch combines three architectures, that is, theophylline aptamer, thiamine pyrophosphate (TPP) aptamer, and twister ribozyme [42]. Each hybrid riboswitch with different communication module sequence (CM) behaves as different logic gates. The relationship of inputs (theophylline, TPP) and ON (lightning bulb) and OFF (dark bulb) of signal (outputs) from respective logic gates are listed. (B) Two- and three-stage controller. In the presence of a metabolite (red disc), the signal is turned on. In the three-stage controller with an added transcriptional amplifier, there is a processing stage between sensing and actuating stages [49], which gives a higher level of signal than a two-stage controller. Abbreviations: TF, transcription factor.

promoter engineering, TF or riboswitch engineering, and adding a transcriptional amplifier between the RNA device and protein actuator [25,46–51] (Figure 2B). For example, improvement of dynamic range has been demonstrated by optimizing the enhancer sequence and number of TF-binding sites [50].

Challenges in Designing Genetic Biosensors

Designing genetic biosensors necessitates lengthy trial-and-error iterations not only for genetic modifications, but also for characterization of the modified microbial hosts [52]. Although remarkable progress has been made in the construction and evaluation of genetic biosensors, there are still a range of challenges in designing genetic biosensors. For example, in microbes, many TFs are responsive to different kinds of metabolites, which offers an opportunity to engineer TF-based genetic biosensors as an HTS tool for metabolic engineering [29]. However, it is still challenging to introduce prokaryotic TFs into eukaryotic hosts to generate functional genetic biosensors [20]. In designing riboswitch-based genetic biosensors, although post-transcriptional regulation by a riboswitch with highly specific ligands can regulate actuation in a simpler way than TF-based biosensors, the functionalities of riboswitches are frequently limited in the aspects of dynamic response and operational range. Furthermore, rational design of riboswitches is still in the developing phase [53–55], partially due to the limited understanding of structure-toggling mechanisms associated to the desired performance.

Another challenge is improving the performance of genetic biosensors, in terms of range and sensitivity. One strategy to overcome this challenge is to engineer existing TFs or riboswitches by directed evolution to improve their binding specificity to a metabolite of interest (Figure 1D). Error-prone PCR-based random mutagenesis coupled with metabolite-specific selection enables specific recognition of effectors by TFs [56–58]. The TtgR regulatory protein from *Pseudomonas putida* was engineered by directed evolution and subjected to iterative fluorescence-activated cell sorting (FACS). Library variants with improved response to resveratrol were selected based on the fluorescence of RFP that correlated with resveratrol production [57,58].

Moreover, the selection and evolution processes for both TF- and riboswitch-based sensing devices are currently still time-consuming. Furthermore, the complexity in signal transduction and the disharmony between a host system and heterologous regulatory devices pose considerable challenges. It is difficult to design a genetic biosensor to precisely real-time monitor natural products secreted into the extracellular environment [59–61]. Given that a biosensor is a powerful tool for real-time monitoring of metabolites and for precise control of the expression of pathway enzymes, there have been recent efforts made to sense natural products secreted into the extracellular environment [62–66]. For example, Chen and colleagues designed a biosensor to monitor extracellular putrescine produced and secreted by *E. coli* [62]. Specifically, a native PuuR repressor responsive to putrescine was chosen for sensing. For signal transduction, a cognate operator was added into the promoter to regulate the expression of GFP. Interestingly, GFP signal in a putrescine producer correlated with the titer of excretory putrescine. This study provides a valuable strategy for using genetic biosensors to monitor extracellular secreted natural products although the mechanism remains unclear. Furthermore, given low or no accessibility to extracellular target metabolites due to cell membrane barrier, use of a G protein-coupled receptor (GPCR) in a genetic biosensor has been reported to detect medium-chain fatty acids (MCFAs). This was accomplished by coupling an MCFA-responsive G protein to a receptor on the cellular membrane, which enabled the transduction of subsequent signals in the presence of extracellular MCFAs [67]. Likewise, Ehrenworth and colleagues engineered a human serotonin GPCR to detect serotonin secreted by a serotonin producer [68]. However, continuous efforts, particularly in developing HTS strategies, are required to overcome these challenges in the designing of

genetic biosensors. Integrative efforts from leading biofoundries such as the recently formed Global Biofoundries Alliance would aid in achieving such a goal [69].

Application of Genetic Biosensors for Natural Product Biosynthesis

TF- and riboswitch-based biosensors have gained significant attention due to their applications for producing natural products. As such, efforts have been put into the development of natural product production, including optimization of biosynthetic pathways, identification of rate-limiting genes, and screening of evolved enzymes and strains (Table 1). In this section, we review the applications of genetic biosensors for the biosynthesis of natural products.

Application of Genetic Biosensors Based on Native TFs

Native sensory proteins have been identified to serve as devices for developing TF-based genetic biosensors to detect intermediates or end products in a biosynthetic pathway. Biosensors have been devised to facilitate optimization of microbial cells factories for producing a range of natural products that have wide applications in industry, such as vitamins, sugars, itaconic acid, *trans*-cinnamic acid, fatty acids, intermediate metabolites, isobutanol, 1-butanol, flavonoids, alkaloids, alcohols, and aldehydes. Furthermore, biosensors have also been exploited to improve enzyme activity [11,12,14,46,51,57,70–77]. A major application of these TF-based biosensors is screening of high production strains. For example, Hanko and colleagues developed an itaconic acid biosensor through expression of a transcription factor ItcR from *Yersinia pseudotuberculosis* (YpItcR) [12]. In this design, the produced itaconic acid subsequently binds to YpItcR and activates RFP expression through a native promoter P_{ccl} . Using this YpItcR/ P_{ccl} biosensor, the concentration of the inducer L-arabinose was optimized to induce the expression of *cadA* gene for itaconic acid production [12]. This study demonstrates the feasibility of improving natural product biosynthesis by using an inducible TF-based biosensor under the control of a native promoter to optimize the induction of pathway enzymes. A similar strategy has been applied to detect and enhance the production of alcohols and aldehydes in *E. coli* [70,75,78]. Moreover, there are also studies on designing biosensors to improve microbial production of flavonoids. Siedler and colleagues developed a QdoR-based biosensor that detected and improved the production of quercetin and kaempferol as well as a FdeR-based biosensor for naringenin. Particularly, the QdoR-based biosensor was able to detect the kaempferol level in real time and improved the production of kaempferol [79]. In addition, Mahr and colleagues constructed a Lrp-based biosensor and screened a *Corynebacterium glutamicum* mutant library for a high L-valine producer. Consequently, it was demonstrated that a loss-of-function mutation in the urease accessory protein ureD contributed to the high production of L-valine in *C. glutamicum* [80].

Native TF-based biosensors have been designed to detect intermediates such as malonyl-CoA and fatty acyl-CoA, which serve as important building blocks for many important natural products. Malonyl-CoA biosensors are mainly constructed by using the transcriptional repressor FapR and tandem copies of its binding sequence FapO. The use of a malonyl-CoA biosensor in *E. coli* and *Saccharomyces cerevisiae* has been shown to increase fatty acid titer by 2.1- and 22-fold, respectively, as compared to the strains without the biosensor [81,82]. Kalkreuter and colleagues developed a new FapR-based biosensor in which the RBS site of FapR was mutated. Compared to its prototypical biosensor, the resulting FapR-based biosensor has shown a higher sensitivity to malonyl-CoA [14]. This study demonstrates the potential of using this biosensor to improve malonyl-CoA-derived natural products such as fatty acids, phenylpropanoids and polyketides.

Similar to malonyl-CoA, fatty acyl-CoA biosensors can be constructed by using the transcriptional repressor FadR and tandem copies of its binding sequence. A fatty acyl-CoA biosensor

Table 1. Production of Natural Products Using TF- and Riboswitch-Based Biosensors

Natural product	Regulatory element	Output	Regulation	Sensing purpose	FC _{signal}	Host	Production level		Refs
							FC	Titer or yield	
TF-based biosensors									
Itaconic acid	<i>Y. pseudotuberculosis</i> /ItrC	RFP	+	Monitoring product	215	<i>E. coli</i>	4.3	102 mg/l	[12]
Fatty alcohol	<i>E. coli</i> /FadR	GFP	+	Monitoring intermediate acyl-CoA	3.3	<i>S. cerevisiae</i>	1.6	11 mg/g DCW	[6]
Adipic acid	<i>E. coli</i> /BenM	GFP	+	Product detection	15	<i>E. coli</i>	NA		[88]
<i>trans</i> -Cinnamic acid	<i>E. coli</i> /HcaR	eYFP	+	Product detection	120	<i>E. coli</i>	3.7	125 mg/l	[51]
Fatty acids	<i>B. subtilis</i> /FapR	GFP	+	Monitoring intermediate malonyl-CoA	12	<i>E. coli</i>	15.7	3.68 g/l	[81]
3-Hydroxypropionic acid	<i>B. subtilis</i> /FapR	tdTomato	-	Monitoring intermediate malonyl-CoA	2.4	<i>S. cerevisiae</i>	22	1.2 g/l	[82]
<i>p</i> -Coumaric acid	<i>B. subtilis</i> /PadR	YFP	-	Monitoring product	130	<i>S. cerevisiae</i>	30	148 mg/l	[78]
3-Dehydroshikimic acid	<i>E. coli</i> /CusR	GFP	+	Screening producer strain	2.5	<i>E. coli</i>	16.7	36.7 g/l	[105]
Ectoine	<i>E. coli</i> /AraC	eGFP	+	Screening rate-limiting enzymes	3.5	<i>E. coli</i>	3.3	10 mg/g DCW	[106]
<i>cis, cis</i> -Muconic acid	<i>Acinetobacter</i> sp. ADP1/BenM	GFP	+	Monitoring product	7	<i>S. cerevisiae</i>	2.9	430.8 mg/l	[104]
L-Valine	<i>C. glutamicum</i> /Lrp	eYFP	+	Screening producer strain	3.4	<i>C. glutamicum</i>	1.96	5.8 g/l	[80]
Tyrosine	<i>E. coli</i> /TyrR	MutD5-mCherry	+	Screening producer strain	50	<i>E. coli</i>	5	16 mg/l	[107]
D-Psicose	<i>A. tumefaciens</i> /PsiR	GFP	-	Product detection	3.5	Cell free	NA		[108]
Benzoic acid	<i>P. putida</i> /BenR	sfGFP	+	Product detection	180	Cell free	NA		[87]
Benzoic acid	<i>E. coli</i> /BenM	GFP	+	Product detection	1.5	<i>E. coli</i>	NA		[109]
Riboswitch-based biosensors									
L-Lysine	<i>B. subtilis</i> /5'-UTR of LysC	tetA-sGFP	+	Screening producer strain	3.5	<i>E. coli</i>	NA	0.6g/l	[89]
L-Tryptophan	Artificial (SELEX)	tetA-sGFP	+	Screening producer strain	3	<i>E. coli</i>	NA		[89]
Naringenin	Artificial (SELEX)	tetA-sGFP	+	Monitoring product	1.9-2.9	<i>E. coli</i>	NA		[7]
Caprolactam	Artificial (SELEX)	tetA-sGFP	+	Monitoring product	3.5	<i>E. coli</i>	NA		[32]
Histamine	Artificial (SELEX)	mCherry	+	Monitoring product	10.9	Cell free/liposome	NA		[110]
Theophylline	Hammerhead ribozyme, artificial device	eGFP	+	Monitoring product	200	<i>S. cerevisiae</i>	NA		[49]

Table 1. (continued)

Natural product	Regulatory element	Output	Regulation	Sensing purpose	FC _{signal}	Host	Production level		Refs
							FC	Titer or yield	
Paromomycin	Artificial (SELEX)	eGFP	-	Monitoring product	8.5	<i>S. cerevisiae</i>	NA		[91]
Adenosyl-cobalamin	<i>E. coli</i> /5'-UTR of BtuB	sfGFP	-	Screening producer strain	>10	<i>E. coli</i>	NA		[13]

+, activation; -, repression.

Abbreviations: DCW, dry cell weight; FC, fold change.

increased fatty alcohol yield by 1.8- and 3-fold in *E. coli* and *S. cerevisiae*, respectively, as compared to the controls [6,76]. In addition, fatty acyl-CoA biosensors were designed by using bacterial FadR transcriptional repressors and yeast synthetic promoters containing the DNA-binding operators for applications in *S. cerevisiae* [83]. This potentially allows for the regulation of protein expression based on the availability of fatty acid intermediates. Collectively, these studies demonstrate the viability of using genetic biosensors derived from native TFs and their binding sequences to detect intermediates of biosynthetic pathways and enhance the biosynthesis of natural products.

In addition, Schendzielorz and colleagues constructed a biosensor pSenLys-Spc which detects L-lysine, L-arginine, and L-histidine. With FACS as an HTS approach, the biosensor was used to screen mutants of respective enzymes (encoded by ArgB, LysC, and HisG) that are less sensitive to their corresponding amino acids. Consequently, overexpression of the improved enzyme mutants increased the production of those amino acids in *C. glutamicum* [84]. This study demonstrates the capability of the TF-based biosensor to improve the biosynthesis of natural products through harnessing feedback control from end products.

Application of Genetic Biosensors Based on Modified TFs

Although a wide range of TFs responsive to a variety of molecules have been identified from nature, it is still a challenge at times to discover a suitable TF for developing a biosensor for a target compound due to nonspecific binding of analytes [85,86], low sensitivity [81], and poor dynamic range [23,25]. However, native TFs can be engineered to broaden or alter their ligand specificity to generate modified TFs for developing biosensors that respond to intracellular or extracellular molecules of interest [11,87]. For example, by improving signal-to-noise ratio and sensing sensitivity, Yeom and colleagues developed an NitR mutant (L117F)-based biosensor, which enabled the detection of lactams such as ϵ -caprolactam and ω -amino fatty acids [10]. In this study, engineering of the TF NitR from *Alcaligenes faecalis* by random mutagenesis and selection by FACS was combined with systematic combinatorial analyses and optimization of promoters and RBSs that regulate the expression of the NitR mutant and GFP reporter. A similar strategy has been used to develop biosensors to detect *trans*-cinnamic acid, malonyl-CoA, adipic acid, *p*-coumaric acid through improved sensing sensitivity, dynamic range, and effector binding specificity [14,51,57,88].

TF-based genetic biosensors have become a powerful tool for strain evolution [75,79], enzyme engineering [73,80], and real-time monitoring of natural products and their intermediates [63]. Although the use of modified TFs can enable development of a wider range of biosensors to facilitate natural product biosynthesis, their use is lagging compared to native TFs. Engineering of modified TFs can be accelerated by deepening our understanding of the regulatory mechanisms of the TFs and using enabling technologies for efficient HTS of TF mutants.

Application of Riboswitch-Based Biosensors

Riboswitches can be obtained from natural genetic sources or an artificial RNA library constructed by SELEX [86]. They could also be combined with other RNA-based architecture to expand their functions and capabilities. Riboswitches are commonly used for monitoring target natural products or their intermediates during pathway or strain optimization [41]. The advantages that they have over TF-based biosensors are that they are encoded by only short DNA sequences and that they are more transferable between microbes given that they function at post-transcriptional level.

As functional roles of noncoding RNA become apparent, applications of native riboswitches for designing genetic biosensors are increasing [85]. For example, Yang and colleagues developed a riboselector that consisted of an L-lysine riboswitch module and a selection module. The native L-lysine riboswitch that controlled the expression of a tetracycline resistant gene was used in a growth-based selection assay to enrich the pathway-optimized high lysine producers. A similar application of the riboselector was devised for the case of L-tryptophan [89]. This study demonstrated application of the riboswitch-based biosensor in expediting the evolution of metabolite-producing strains. In another study, the *glmS* ribozyme, which can respond to intracellular glucosamine 6-phosphate (GlcN6P), was used to construct a suicide riboswitch. The *glmS* ribozyme was applied through the HTS of *S. cerevisiae* GFA1 mutant strains that produced a higher amount of GlcNAc [90].

Genetic biosensors based on artificial riboswitches have been developed and applied in the detection of natural products. For example, by using SELEX and *in vivo* screening, Jang and colleagues developed an artificial riboswitch that can detect caprolactam. The selected riboswitch showed a 3.4-fold higher specificity to caprolactam and was able to distinguish between caprolactam and other tested lactams such as butyrolactam and valerolactam [32]. In another study, an artificial biosensor was developed by using SELEX to detect naringenin. The selected riboswitches showed up to a 2.9-fold increase in activated gene expression. Specifically the riboswitch Mid3 was able to distinguish between naringenin and another flavonoid, eriodictyol, when simultaneously produced in *E. coli* [7]. Boussebayle and colleagues developed an artificial paromomycin riboswitch through Capture-SELEX strategy where ligand immobilization was excluded [91]. Furthermore, Felletti and colleagues developed a hybrid riboswitch by combining three architectures, that is, theophylline aptamer, TPP aptamer, and twister ribozyme. The hybrid riboswitches acted as one- and two-input regulators that could regulate gene expression in *E. coli* and *S. cerevisiae* [42] (Figure 2A). In another study, a riboswitch-based genetic biosensor that enabled self-directed evolution (RiDE) was developed to acquire acid tolerance phenotype in *E. coli* [92]. This study demonstrated the potential of using RiDE to isolate strains that are suitable for producing highly acidic natural products, such as organic acids, or screening of the involved enzymes.

Although the application of riboswitch-based biosensors for the biosynthesis of natural products has been expanding, this approach is still in its infancy and it is much less commonly used than that of TF-based biosensors. To date, riboswitch-based biosensors have been mostly used for detection of target natural products but rarely for improving the production, mainly due to a narrower dynamic range and limited kinds of detectable chemicals compared to TF-based biosensors (Table 1). Nevertheless, with advances in HTS technologies, screening and discovery of riboswitches will be greatly accelerated to promote the development of more riboswitch-based biosensors.

Concluding Remarks

The design and application of genetic biosensors for the biosynthesis of natural products have made significant progress in the past decades. Genetic biosensors offer a promising tool for

Outstanding Questions

How can we design a genetic biosensor for sensing a specific metabolite with high specificity, sensitivity and a broader dynamic range?

How can we design a functional genetic biosensor using a heterologous transcription factor in a new microbial host?

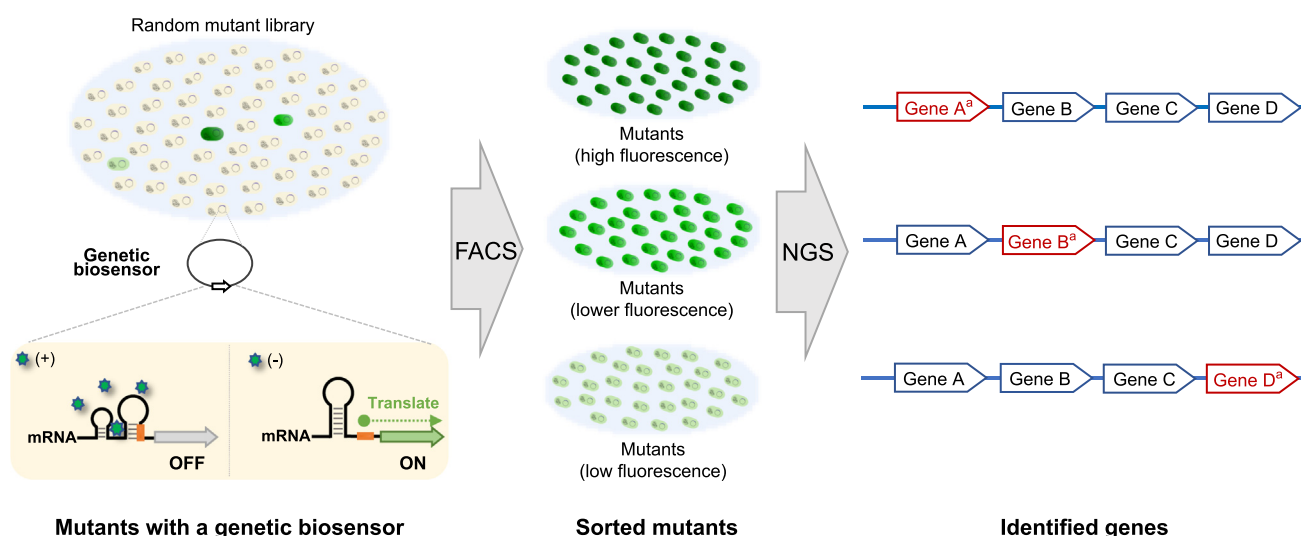
How can we design a genetic biosensor that can rapidly and precisely detect extracellular natural products?

How can we design a riboswitch-based biosensor that can effectively improve natural product biosynthesis?

screening enzymes and metabolic pathways, which can expedite the process of engineering microorganisms. There are several challenges underlying the design and application of genetic biosensors. Continuous efforts are essential to improve and expand the approaches for rapid designing of genetic biosensors, design genetic biosensors with desired functions, for example, dynamic range, sensitivity and substrate specificity, and develop multigene-controlling genetic biosensors.

The following strategies can be used to facilitate the designing and application of genetic biosensors. First, the recent wealth of bioinformatics provides the opportunity to further and expand our understanding of regulatory networks and metabolic pathways and to expedite the discovery of native genetic parts from nature as well as the rational design of artificial genetic parts. This would aid in shortening the process of genetic parts selection and biosensor construction. Second, combination of TFs and riboswitches that can complement each other have emerged. This is exemplified by Wang and colleagues who reported a hybrid controller consisting of a riboswitch-based detector and a protein regulator for compensating the low dynamic range of a riboswitch [49]. Third, use of feedback control offers solutions for controlling cell population, reducing metabolic burdens, and controlling expression of multiple genes using one input signal [93–95]. Ceroni and colleagues reported a dCas9-based feedback-regulation system in which the promoter automatically adjusts the downstream gene expression in response to burden [94]. In order to control multigene expression by one chemical signal, Cunningham-Bryant and colleagues reported a genetic controller that consists of catalytically inactive Cas9 (dCas9) and RNA-binding protein fused to a chemically inducible TF [95].

Furthermore, the combination of genetic biosensors with biofoundry-based automation and high-throughput analytics could be a key strategy to expedite the process of systematic elucidation and screening of biological systems (Figure 3). This is exemplified by RiboFACSeq, which combined riboswitch-based biosensor with FACS and NGS [13]. HTS combined with co-cultivation of *Bacillus subtilis* and *E. coli* sensor cells inside nanoliter reactors was also reported for vitamin



Trends in Biotechnology

Figure 3. Combination of a Genetic Biosensor with High-Throughput Fluorescence-Activated Cell Sorting (FACS) and Next-Generation Sequencing (NGS) for the Biosynthesis of Natural Products. A genetic biosensor generates fluorescence when the target natural product (star) is absent. A library of random mutants that carry the genetic biosensor are generated and sorted by high-throughput FACS according to fluorescence generated (i.e., high, lower, or low), followed by NGS to identify the genes (e.g., Gene A^a, Gene B^a, or Gene D^a) potentially involved in the biosynthesis of the natural product.

B2 production [96]. In addition, use of cell-free systems can speed up systematic elucidation and screening of biological systems [97]. Furthermore, deep learning and integration of omics data is essential to expedite the process of genetic biosensor design through biofoundries [98]. With these continuous efforts, designing and applying genetic biosensors for industrial applications will soon be a mainstream strategy for improving the biosynthesis of natural products in engineered microbial hosts (see Outstanding Questions).

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