# Recent Developments in Synthetic Biology Toolbox

7

Priyanka Patel Vatsa, Manju Shri, Poonam Bhargava, Chaitanya G. Joshi, and Madhvi Joshi

**Abstract**

Synthetic biology is a new and evolving branch of science, which has extensive application in the surrounding as well as human life. This field deals with the knowledge gain from the living system by the means of bioinformatics or other relevant field and try to regulate or restructure the pathways and the system of the higher organism in much simpler microorganism. It actually translates the knowledge gain from an organism or system in simpler system for the benefit of the nature. Evolution in the field of biotechnology, easy fast and high-throughput and accurate technologies available for DNA sequencing, and synthesis, has made it very easy to design and structure a specific pathway, which is useful in a particular organism in a model organism, which in field of synthetic biology is termed as chassis. Synthetic biology brings together different areas such as engineering, molecular biology, cell biology, biotechnology, bioinformatics, and system biology in such a way that they all together forms this new area with vast application in various fields. Synthetic biotechnology has its application in almost all fields such as from cellular programming to drug designing to biofuels production. In this chapter, we will be mainly dealing with the application part of the synthetic biotechnology in development of biosensors, for new drug discovery, tools for application in agriculture, for secondary metabolism, for chemical production, and for biofuel production.

P. P. Vatsa · M. Shri
Gujarat Biotechnology Research Center, Department of Science and Technology, Government of Gujarat, Mahanagar Seva Sadan Building (MS Building), Gandhinagar, Gujarat, India

P. Bhargava · C. G. Joshi · M. Joshi (✉)
Gujarat Biotechnology Research Center, Department of Science and Technology, Gandhinagar, Gujarat, India
e-mail: jd1-gbrc@gujarat.gov.in



V. Singh et al. (eds.), *Engineering of Microbial Biosynthetic Pathways*,
https://doi.org/10.1007/978-981-15-2604-6_7

**Keywords**

Synthetic biology · Bioengineered yeast · Biosensors · Biofuels

## 7.1 Introduction

Synthetic biology is an emerging field which deals with the regulation, synthesis as well as reprogramming of genes as well as various cellular metabolic pathways either in the same host or manipulating and constructing new pathways in heterologous host. However, sometimes it also represents and construct new genetic circuits, or enzymes even new biological entities. SB brings together different areas such as engineering, molecular biology, cell biology, biotechnology, bioinformatics, and system biology in such a way that they all together forms this new area with vast application in various fields. The basic difference between SB and molecular biology along with cell biology is that it relies more specifically on understating and focusing on the small components or important steps in metabolic/cellular pathways that can be synthesized, modeled as well as can be used for the assembly of whole pathway with desired control over new synthesized pathway. SB also assembles small parts (of biological origin and system) and devices (engineering side) into a larger system for solving a specific problem. It is an application-driven field endeavoring to put on a cogent engineering methodology to restructure the biological systems, for producing treasured and innovative biological functions. It can be assumed as a natural evolution of biotechnology as opposed to being a separate field although it does offer a novel and exciting approach. As a comparatively new field, it sits at the intersection of engineering principles and biological design. The overall aim of the synthetic biology is to gain a better understanding of coordinating and regulating the genes as well as pathways in living cells (Andrianantoandro et al. 2006). For expression of a gene, various elements such as promoter, transcription factor binding sites, and terminators are necessary and important. However, if expression of a gene is to be done in a heterologous host or just the regulation of gene in the same host is required, then a different component or element that is vector also comes in the picture, which is also very important. In particular, the engineering of synthetic systems involves practical and rationale design of disparate synthetic parts that all must work together to obtain the desired function.

Synthetic biology has two main approaches "top-down" and "bottom-up." In the top-down approach, an existing functional cell or organism sometimes referred as biology is modified or reengineered, while in the second bottom-up approach, synthesis of functioning circuits and pathways occurs with the help of DNA and other biomolecules. The second approach starts from the scratch and ends in the final synthesis of a complex pathway or gene cluster. Top-down approach was predominated in the early stages of this field, which allowed scientists to test designed biological circuits in a "chassis." Here in SB, the term chassis is used for a cell or organism (functioning and self-replicating) in which the engineered DNA or biopart is entrenched for producing the desired device or system, e.g., *E. coli*. Utmost top-down biology experiments can be categorized into two ways. The first

one is by combining short elements of a system from various organism in a single cell or organism (modified or engineered organism) to perform a desired task, while in the other way the complex system of an organism or cell is simplified (Fig. 7.1).

Latest progresses in SB have provided various tools for specific regulation of expression of gene of interest, by synthetically engineering promoters for specific genes along with posttranscriptional modification and either degradation or stabilizing the product (Lee et al. 2016). Synthetic biotechnology has its application in almost all fields such as from cellular programming to drug designing to biofuel production. In this chapter, we will be mainly dealing with the application part of the synthetic biotechnology toolbox in the development of SB tool for biosensors, for new drug discovery, tools for application in agriculture, for secondary metabolism, for chemical production, and for biofuel production.

## 7.2 Development of Synthetic Biology Tool for Biomonitoring/ Biosensors

The term "biosensor" or biomonitors denotes a wide variety of devices with the biological common component providing recognition of a certain highly specific target analyte, and converting this detection in an easily detectable, quantifiable response which can be fed to an electronic device for signal processing, data storage, etc.

### 7.2.1 Types of Biosensors

The biological component in many biosensors is either an enzyme, antibody, or whole cell. Based on various biological components, biosensors can be categorized into various classes such as whole-cell biosensor, transcription factor-based biosensors, nucleic acid-based biosensors, and transcription-independent protein-based biosensors (TIPBS). These biosensors on the basis of their construction and organization methods can be further subdivided, which will be covered in the following section.

#### 7.2.1.1 Whole-Cell Biosensor (WCB)

Whole-cell biosensor (WCB) sometimes also referred as bioreporter (Belkin 2003) in which living cell is used as a biological component. These cells have the ability to detect the target analyte via receptors and are able to generate a detectable response, most commonly by inducing expression of a reporter gene. This device uses the whole prokaryotic or eukaryotic cell (experimentally modified for increased transducer capacity or sensitivity toward analyte) as a single reporter, integrating both bio-receptor and transducer elements into the same cell.

If we are using WCB, we can perform two types of bioassays, i.e., turn off and turn on assays (Belkin 2003). In turn off assays, the sample toxicity can be estimated from the degree of inhibition of a cellular activity, or a decreased expression of specific reporter gene. In the second assay that is turn on assay, a promoter of a

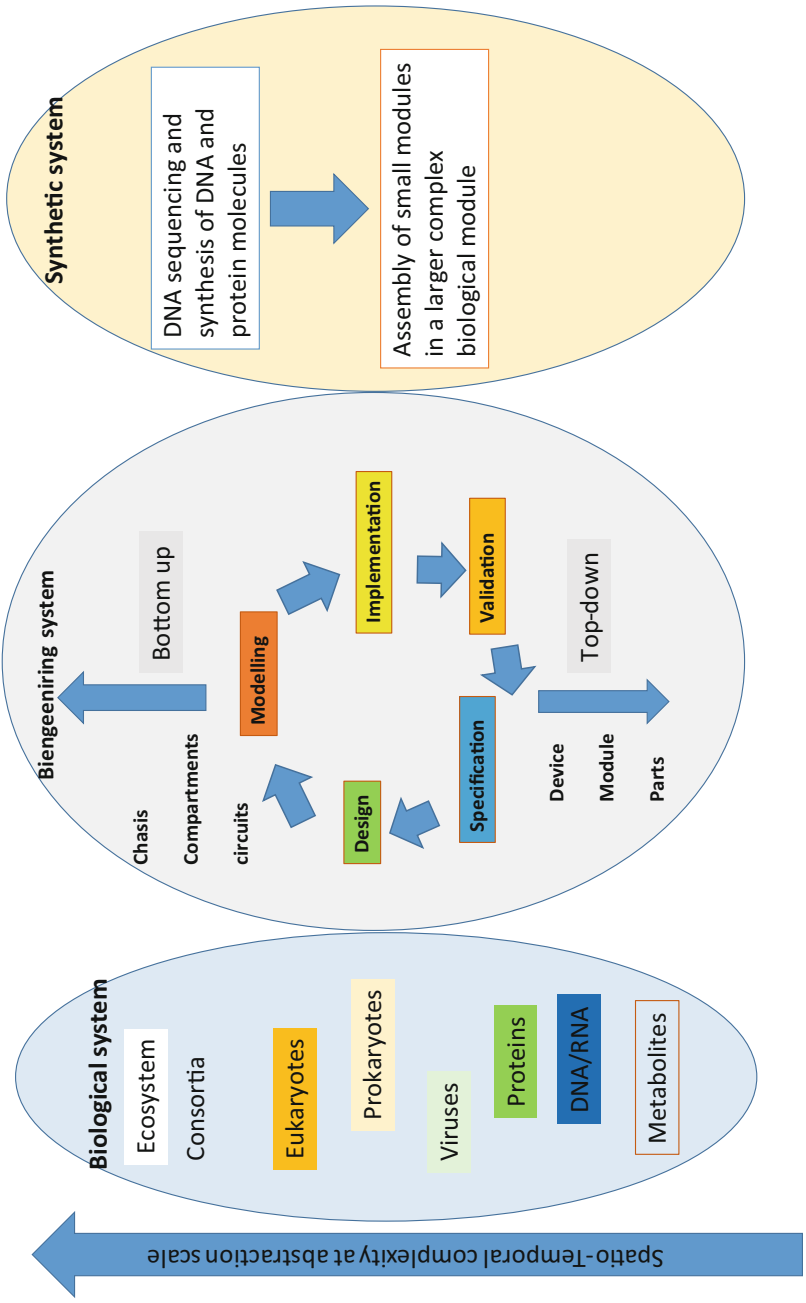


**Fig. 7.1** Different component of synthetic biology showing top-down and bottom-up approach (Ph.D. Thesis: Henary Poicare)

specific gene (which is specific to a given pollutant or target analyte) is fused with the reporter gene. So in this assay the degree of expression of reporter gene is directly proportional to the presence of analyte or pollutant. By comparing both of these assays, we can say that turn off assays are more nonspecific because the signal decreases as a result of a broad range of cytotoxic effects, while turn on assays, based on an inducible gene expression, are usually more specific as induction of the gene reporter only takes place when the pollutant is present. Their specificity will therefore depend on the degree of the gene promoter specificity to be opened by an exclusive pollutant of a chemically related group of pollutants. With respect to specificity, WCBs can be divided into effect- and compound-specific sensors (Yagi 2007). Changes in a physicochemical condition (e.g., pH, temperature, or osmotic changes) or pollutants that give rise to a specific type of toxicity (e.g., oxidative stress or protein damage) stimulate effect-specific biosensors. Compound-specific biosensors respond to only one type of pollutant or compounds with similar chemical features (e.g., any heavy metal).

There are two types of biosensors based on their expression level. One with constitutive expression system, while the other one is with inducible system. A constitutive system comprises a promoter of a specific gene which has higher expression under normal condition, which results in a high basal expression level of the reporter gene. During exposure to the signal (target analyte), the basal expression level of reporter gene is decreased, so the reduction in expression of reporter gene is inversely proportional to the concentration of analyte. In second type of biosensor, inducible biosensors inducible promoter is fused with the reporter gene. So when there is an analyte, its presence will induce the expression of the reporter gene. For such type of biosensors, it is essential to have promoter which is specific for the analyte which needs a detailed study of the promoters.

#### 7.2.1.2 Transcription Factor-Based Biosensors

Transcription factor-based biosensors (TFBs) respond to the ligand by utilizing the transcriptional and translational machinery of cell for the generation of output signals. The ligand binding to the biosensors causes changes in confirmation of the TFBs thus disturbing its binding with the promoters of the gene and finally the expression of specific gene is converted into the output signal. These TFBs further subdivided into the following groups.

##### Native Transcription Factor-Based Biosensors

If the transcription factors, which are native to their origin and not synthetically designed, are used for the development of TFBs, then this system is referred as native transcription factor-based biosensors. These are easy to construct and therefore widely used in the field of synthetic biology for para-hydroxybenzoic acid (Williams et al. 2017), NADP/NADPH (Siedler et al. 2014), muconic acid (Leavitt et al. 2017), fatty acids (Zhang et al. 2012), etc.

##### Heterologous Species Transcription Factor-Based Biosensors

If the host does not have the specific transcription factor for the specific target ligand, the transcription factor of different species can be used for the generation of TFBS.

However, these systems are referred as heterologous species transcription factor-based biosensors. For example, Dietrich et al. (2013) developed a biosensor for butanol which could be used in *E. coli*. This device uses a sigma factor (BMOR) and promoter sequence (PMBO) of *Thauera butanivorans* (Dietrich et al. 2013) and used tetracyclin-resistant-GFp fusion gene (TETA-GFP). PBMO, the promoter sequence, was placed at the 5 prime end of tetracyclin-resistant-GFp fusion gene, while BMOR gene was expressed under its own promoter (PBMOR promoter) (Dietrich et al. 2013). When there is butanol, it binds with BMOR and this ligand gene product (butanol-BMOR) causes activation of PMBOR, and it will further result in increased expression of TETA-GFP. When there is limited supply of butanol, there will be limited expression of GFP fusion protein in comparison to the condition when there is plenty of supply of butanol resulting in excessive expression of GFP fusion protein.

#### Modular Transcription Factor-Based Biosensors

If we are utilizing modular, transcription factors for the generation of TFBS, these devices will be named as modular transcription factor-based biosensors. These are a little bit tricky in engineering comparing to the TFBS discussed above and are more versatile. In this case, we have to take care of the various domains of the protein which must bind to ligand as well as induce transcription of specific gene. The domains either can be fused with transcriptional activation domains and DNA-binding domains or can be expressed. In the absence of the ligand, these domains are disconnected resulting in lesser or limited transcription of the output gene. However in a condition when the ligand is present, the domains are presented at the promoter resulting in increased expression of the target gene (Carpenter et al. 2018).

This approach was utilized by Chou and Keasling (2013) to engineer isopentenyl-pyrophosphate (IPP) biosensors for use in *E. coli* and *S. cerevisiae*. In this biosensor, the araBAD promoter (PBAD) and its native transcriptional regulator Ara C were used in *E. coli*. In its native perspective, when arabinose is present, it binds with AraC, resulting in a conformational change that results in its association with PBAD and induction of transcription of the gene. However, in case of the abovementioned biosensor, the DNA-binding domain of AraC is joined via a linker to a known IPP-binding domain that is IPP isomerase (Idi). Due to crystallographic data, it was assumed that in the presence of IPP, dimerization of IPP-binding domain occurs. In a condition when there is low concentration of IPP, the AraC DBD-Idi fusion protein is free to bind to PBAD resulting in the expression of mCherry-encoding reporter gene. However, in a different scenario when the concentration of IPP is higher, it causes dimerization of Idi and thus blocking the ability of the AraC DBD from inducing transcription from PBAD, and reducing the expression of mCherry.

### 7.2.1.3 Nucleic Acid-Based Biosensors

This is a different class of biosensors, which utilizes the nucleic acids (DNA/RNA) as a functional unit or compartment of the biosensors. These nucleic acid sequences have binding affinity toward target ligand. The binding of the target ligand to the

nucleic acid sequence (DNA/RNA) alters its structure and conformation which is utilized by various mechanisms to generate an output signal (Carpenter et al. 2018).

### Aptamer-Based Biosensors

Aptamers are single-stranded DNA or RNA molecules having affinity toward target ligand (Tuerk and Gold 1990). In general, the binding of the aptamer to the target ligand brings a conformational change in the aptamer. In some of the cases the conformational change results in secondary structure change which could be either from a linear to stem-looped structure, or from a hair-pin to ligand-coordinated structure. The sutural changes in the aptamers upon binding with the ligands are used to generate the signals.

In one example, McKeague et al. (2014) used an DNA aptamer for Ochratoxin A (OTA) for the generation of aptamer-based biosensor. This DNA aptamer has a predominantly stem-looped structure which is modified via ligand binding (Song et al. 2008). In their biosensor, the approach that they had followed depends on the signals that are generated by binding of DNA to the syber green. In a condition when there was no OTA, the aptamer will remain in stem loop structure thus having various sites for the binding of the syber green generating a high signal in the form of fluorescence. However, in presence of OTA, there is conformational change in the aptamers resulting in lesser sites available for syber green binding thus finally reducing the fluorescence.

### Riboswitch-Based Biosensors

Riboswitch is a regulatory segment of a messenger RNA molecule that in the presence of specific small molecule or ligand results in a change in production of the proteins that are encoded by the mRNA. These are composed of two domains, one which has binding site with the ligand while the second domain is a response domain which is used for the generation of signals after binding to ligand (Findeiss et al. 2017). In the presence of ligand, which binds with the binding domain of riboswitch, conformational or structural changes in the domain is induced which further result in the change in response domain resulting in induction of signals. Most of the time, the response domain is a messenger RNA (mRNA) transcript, and the change in structure leads to either activation or reduction of gene expression/translation (Findeiss et al. 2017).

Muranaka et al. (2009) developed thiamine pyrophosphate (TPP) riboswitch developed for *E. coli*. In this particular case, the ligand binding domain (TPP-binding domain) is located at 25 bp upstream of the RBS of tetracycline resistance (TETA) gene. After transcription, the TPP-binding domain folds back onto the RBS and blocks access of the ribosome, preventing translation of TETA. However, in the presence of TPP, the TPP-binding domain binds with the TPP making RBS free for the access of ribosome thus resulting in transcription and translation of TETA, and the *E. coli* containing this biosensor are allowed to grow in the presence of tetracycline.

#### 7.2.1.4 Transcription-Independent Protein-Based Biosensors (TIPBs)

This class of biosensors are very wide in terms of their working, i.e., how they detect an analyte and how they produce the output signals. Main components of a type of biosensors include both receptor and response domains. The analyte is received by the receptor domain, and the signal is produced and transmitted by the response domain (Carpenter et al. 2018).

##### Integrated Transcription-Independent Protein-Based Biosensors

These are the biosensors in which receptor domain/s, which has binding site/capacity to specific ligand, are expressed as a fusion protein with either little or no linker domain to a response domain. In these biosensors when ligand binds to the receptor domain, it causes conformational change in the respective domain which is directly transmitted to the response domain. Then response domain leads to the induction of signals (Stein and Alexandrov 2015).

Nagai et al. (2001) had developed integrated TIBP-based biosensor for calcium utilizing expression of GFP-fused calmodulin. In this biosensor, GFP was expressed with both calmodulin and M13 domains which has binding sites for the calcium. In the absence of calcium, the GFP is not correctly folded due to the disturbance resulting from the calmodulin and M13 domain. However, in the presence of calcium, both of these domains M13 and calmodulin bind with the calcium thus allowing the correct folding of GFP which results in the production of signals in the form of fluorescence.

##### Semi-Modular Transcription-Independent Protein-Based Biosensors

These are the biosensors in which there is a linker present in between the receiver and response domain. In these types of biosensors, the conformational changes in the receiver domain after binding with the ligand is transmitted to the response domain through linker resulting in the induction of signal.

### 7.2.2 Applications of Biosensors

Biosensors are a major application of synthetic biology. They have their application in human heath as well as they can also be used for the detection of any type of heavy metals or pollutant in any type of the sample, which could be either water or soil. Their ability to detect specific and accurately a wide range of molecules makes them highly relevant to a choice of industrial, ecological, medical, and scientific applications.

#### 7.2.2.1 Biosensors for Detecting Heavy Metals Pollutant from Various Samples

Heavy metals are those metals which possess specific gravity either five or five times more than that of water. These are among the most abundant, toxic, and persistent inorganic environmental pollutants (Hill 2004). Human activities such as mining and other industrial anthropogenic activities are responsible for increasing the heavy

metal pollution in the environment. Heavy metals cause their toxicity by increasing reactive oxygen species production. Further, these species (ROS) produced are responsible for significant alterations in nucleic acids, proteins, and lipids (Leonard et al. 2003) which ultimately can result in cell death by necrosis or apoptosis.

Wan et al. (2019) engineered and optimized ultrasensitive *E. coli*-based biosensors for detecting arsenic and mercury contamination in water. This group had developed a modular cascaded signal amplifying methodology and combined this methodology with basal background tuning approaches. By this approach, they were able to improve the sensitivity and output dynamic range of cell-based sensors. The camera of cell phone could also capture the output signals that are produced by the biosensors. This feature made this biosensor portable, handy, and it also decreased the cost of the biosensor.

#### Biosensors for Diagnostic and Detection Devices

In many Gram-negative bacteria, cell-to-cell communication system (quorum sensing) is maintained or established by acyl-homoserine lactone (acyl-HSL) and it results in biofilm production. Research in acyl-HSL-based quorum sensing is achieved by simple methods devised to detect AHLs with the help of bacterial biosensors that recognize exogenously produced AHLs and produce output signals (Steindler and Venturi 2007). For example, Wen et al. (2017) designed a modular DNA-encoded biosensor in cell-free protein expression systems that can be used to detect a bacterial biomarker of *Pseudomonas aeruginosa* infection from samples of cystic fibrosis patient. This pathogen produces two types of the biomolecules (AHLs), *N*-butyryl-homoserine lactone (C4-HSL), and *N*-3-oxo-dodecanoyl-homoserine lactone (3OC12-HSL). With the help of this specific biosensor, *Pseudomonas aeruginosa*-specific QS molecules can be detected in patient samples (sputum, urine and blood).

## 7.3 Development and Application of Synthetic Biology Tool in Agriculture

The application of synthetic biology in the field of the agriculture has some serious challenges. These challenges include genome size of the plants (wheat, a hexaploid >15 Gb genome), ploidy level of the crop, propagation, transformation of plants that is still very difficult. CRISPR/Cas9-mediated gene editing (Puchta 2016) has boosted the field of plant biotechnology. It is also important for primary industries dealing with genetic manipulation of food crop to take prior permission from the government; as well, they should also aware of consumer attitudes (Goold et al. 2016).

Improvement of the nitrogen fixation in the field by the plants can be achieved by various applications of synthetic biology (Rogers and Oldroyd 2014). While plants are unable to fix atmospheric nitrogen. However, the microbes are capable of fixing atmospheric nitrogen, particularly rhizobia in legumes. By engineering the plant microbiome, the nitrogen fixation in crop-associated microbial species can be

improved (Rogers and Oldroyd 2014). Similarly, considerable efforts are underway to introduce direct nitrogen fixation into higher plants (Allen et al. 2017) and to introduce novel symbiotic associations with nitrogen-fixing bacteria (Rogers and Oldroyd 2014). Photosynthesis is a very unique feature of the plants that also drives agriculture and has numerous opportunities for plant synthetic biology. RubisCo which is the key enzyme (responsible for $CO_2$ fixation) of the photosynthesis is inherently inefficient, with a theoretical maximum efficiency of ~11% but typically not exceeding a few percent (Ort et al. 2015). However, the introduction of carboxysomes of cyanobacteria into the chloroplast could potentially overcome the inherent suboptimal activity of RuBisCO (Giessen and Silver 2017). Thus the capacity and efficiency for fixation of atmospheric $CO_2$ can be achieved by synthetic biology ultimately increasing the agricultural productivity.

On the other side, the nutritional value of the foods can also be stimulated such as development of carotenoid-enriched functional crops and oilseed crops with increased levels of omega-3 fatty acids. The carotenoid profile of tomato seeds has been shown to be modified by altering the plastids (D'Andrea et al. 2018). This study had shown that this pathway could be manipulated to breed fruit crops with designed carotenoid content. The synthetic control of plastid identity is an ambitious approach that has been proposed to develop new carotenoid-enriched crops (Llorente et al. 2017). The nutritional value and quality of seeds of canola-producing oils have been improved by metabolic engineering. *Arabidopsis thaliana* (a model plant) had been shown to enhance the quality of seed oil by using seven enzymes from five different organisms, the yeasts *Lanchancea kluyveri* and *Pichia pastoris* and the algae *Micromonas pusilla*, *Pyramimonas cordata*, and *Pavlova salina* (Petrie et al. 2012).

Land usage is also an increasing problem for agriculture, which also can also be solved by the application of synthetic biology in the same field. As population is increasing day by day, limiting the availability of land for agriculture. Along with the population industrialization and increasing pollution is also responsible for reducing the available land for the agriculture. Two potential strategies to address this issue are bioremediation using microbes, and engineering plants to grow in non-arable land. The first, bioremediation, is a technique in which the ecological system including microbes is modified to change the environment. Extension of this concept to rational engineering approaches pertinent to agriculture is also being undertaken by researchers to convert microbes such as *S. cerevisiae* and *E. coli* into potential bioremediation agents (Ravikumar et al. 2017). These are capable of bioremediation of heavy metal contamination, degradation of toxic aromatic compounds, and biomass-based sugars. However, in the second strategy the plant itself is genetically modified so that it can grow in unfavorable condition. This is possible by the sequencing of different cultivars as well as the organisms or the plants growing in the extreme condition. Reverse engineering of traits such as arsenic accumulation and tolerance gene of aquatic plant *Ceratophyllum demeresum* into crop plants such as rice had shown remediation in areas of the world affected by heavy metal stress, and potentially will aid in the regeneration of non-arable land (Shri et al. 2014). A comprehensive strategy to harness the potential of synthetic biology will deliver the next-generation of improved agricultural crops.

## 7.4 Development of Synthetic Biology Tools for Secondary Metabolites Production

Secondary metabolites are the intermediates of the primary metabolism of the cells. These molecules do not play very important role in the primary metabolism. However, these molecules possess various biological activities such as antimicrobial, antifungal, and antiviral activity. These molecules are also responsible for immune defense system of plants. Secondary plant metabolites are classified according to their chemical structures into several classes such as phenolics, alkaloids, terpenes, lipids, and saponins. Microbial synthesis overcomes many of the obstacles hindering traditional chemical synthesis and plant metabolic engineering, thus providing an alternative avenue for exploring plant specialized pathways. In the coming section, we are going to discuss synthetic biology tools for various secondary metabolites production.

### 7.4.1 Production of Lipids Via Synthetic Biology

Improvements in synthetic biology and enabling technologies like sequencing as well as synthesis of DNA and analytical techniques have speeded up the cycles for protein and metabolic engineering to that extent where these can be organized for the biosynthesis of a particular molecule.

Lipids extracted from plants have various applications such as the cocoa tree (*Theobroma cacao*), which is utilized for industrial purposes. The lipids extracted from this plant are the basic component of cocoa butter. The main composition of this triacylglycerol product is 1-palmitoyl-3-stearoyl-2-oleoylglycerol, 1,3-dipalmitoyl-2-oleoylglycerol, and 1,3-distearoyl-2-oleoylglycerol. Wei et al. (2018) cloned and expressed the biosynthesis gene of TAGs (glycerol-3-phosphate acyltransferase, lysophospholipid acyltransferase, and diacylglycerol acyl transferase) from *T. cacao* into *S. cerevisiae* through Gibson assembly. Table 7.1 represents few secondary metabolites that are synthetically produced in engineered host.

### 7.4.2 Alkaloids

Alkaloids are known to have various roles in human health, ranging from the relief from pain (opioids) to treatment of a disease like cancer (vinblastine and vincristine). Opioids belongs to an important class of medicines including morphine and codeine. Morphine is a pain killer, member of opioids synthesized naturally by various animals and plants. It actually works on central nervous system of patient and decreases the sensation of the pain. Synthetic biology can be helpful in the overproduction of such type of pharmaceutically important molecules as we have the sequence of the genes of morphine and other specific alkaloid molecule. If we are able to overproduce even a molecular intermediate of a metabolic pathway, it can change the flux of the system and have a capacity to mold the flux toward the higher

**Table 7.1** Secondary metabolites produced in engineered host (Cravens et al. 2019)

| Compound | Titer | Engineered host | Utility |
|---|---|---|---|
| Taxadiene | 1 g/L | *E. coli* | Precursor of taxol (widely used as anticancer drug) |
| Strictosidine | 0.5 g/L | *S. cerevisiae* | Monoterpene indole alkaloid PNP branch point |
| Reticuline | 160 mg/L | *E. coli* | Alkaloid PNP branch point |
| Naringenin | 110 mg/L | *E. coli* | Flavonoid PNP branch point |
| Amorphadiene | >40 mg/L | *S. cerevisiae* | Precursor to antimalarial drug (artemisinin) |

**Table 7.2** Engineered strain for biosynthesis of reticuline

| Module | Name of module | Purpose for synthesizing modules/components of modules |
|---|---|---|
| Module 1 | Precursor overproduction module | Increase accumulation of L-tyrosine and 4-hydroxyphenylacetaldehyde (4-HPAA) |
| Module 2 | Tetrahydrobiopterin module | Expression of four proteins from *Rattus norvegicus*—(1) sepiapterin reductase, (2) 6-pyruvoyl tetrahydrobiopterin synthase, (3) quinonoid dihydropteridine reductase, and (4) pterin carbinolamine dehydratase |
| Module 3 | (S)-norcoclaurine module | Expression of four proteins: (1) mutant of tyrosine hydroxylase; (2) catecholamines and the dihydrofolate reductase both from *R. norvegicus*; (3) DOPA decarboxylase from the bacteria *Pseudomonas putida*, and (4) norcoclaurine synthase from the plant *Coptis japonica* |
| Module 4 | (S)-reticuline module | For the expression of five plant proteins: (1) norcoclaurine 6-*O*-methyltransferase, (2) coclaurine-*N*-methyltransferase, (3) 4′-*O*-methyltransferase, (4) cytochrome P450 reductase (1–4 from *P. somniferum*), (5) *N*-methylcoclaurine hydroxylase from *Eschscholzia californica* |

production of the desired compound. One such type of example is a yeast strain produced by Galanie et al. (2015) that can produce (S)-reticuline, a key biosynthetic intermediate of various downstream benzyl isoquinoline alkaloids including the morphine. This strain was developed by customized genetic modules with better carbon flux through tyrosine to (S)-reticuline. In this modified strain, the biosynthetic pathway of reticuline had been divided into four genetic modules containing 17 biosynthetic enzymes. Details of the modules are described in Table 7.2.

### 7.4.3 Nonribosomal Peptides (NRP)

Nonribosomal peptides belong to a class of peptide secondary molecules. These are primarily synthesized by microorganisms. NRP synthetases (NRPSs) is the enzyme

which is utilized for the synthesis of the NRPs. This particular enzyme differs from ribosome in the action of production of peptides as it does not depend on mRNAs. These enzymes are very specific ass one type of enzyme is responsible for the production of specific NRPs. So if we change the structure of NRP synthetase, we can produce an entirely different NRPS. Awan et al. (2016) expressed the whole biosynthetic pathway for benzylpenicillin synthesis in the yeast *S. cerevisiae*. The biological pathway for benzylpenicillin synthesis consists of five enzymes (encoded by pcbAB, npgA, pcbC, pclA, and penDE) (Fig. 7.2).

### 7.4.4 Flavonoids

Flavonoids consist of a variety of application in health practices. They possess various activity including antioxidant and antimicrobial activities (Skrovankova et al. 2015). Breviscapine is a flavonoid which is extracted from the vegetal tissues of *Erigeron brevisca* and is used in Chinese medicine. The major components of breviscapine are apigenin 7-*O*-glucuronide and scutellarin. Liu et al. (2018) group was able to produce apigenin 7-*O*-glucuronide and scutellarin from glucose in yeast.

## 7.5 Development of Synthetic Biology Tools for Biofuel Production

*Biofuels* are fuels that are produced from biomass, rather than a fuel which is produced by geological processes involved in the formation of oil. The production of biofuel is less time and energy consuming in comparison to fuels that are produced by geological activities. As time passes on, the extraction of fuels from the natural resources are decreasing, so there is a need of looking into other options also for the production of fuels. Here comes the synthetic biology which provides various model organisms such as *Escherichia coli* microalgae and *Saccharomyces cerevisiae* for the production of biofuels. In this section, we will discuss the application of yeast and microalgae for biofuel production as well as the problems associated while using yeast and microalgae for the same.

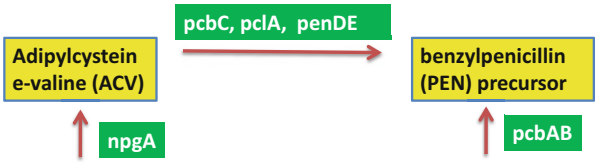


**Fig. 7.2** Schematic diagram showing various genes involved in biosynthetic pathway for benzylpenicillin synthesis in the yeast *S. cerevisiae* by Awan et al. (2016)

### 7.5.1 Synthetic Biology Tools for *S. cerevisiae* for Biofuel Production

#### 7.5.1.1 DNA Assembly Tool

A very first step in SB is the assembly of DNA sequence of biological pathway into an expression cassette. To complete this goal, a very quick and highly efficient method which is competent of linking genes in a particular pathway in a long chain is required. As in a pathway, various genes are under the control of various promoters and terminators exist, so the method must offer a very high capacity and efficiency so that a very long nucleotide sequence with various modules can be combined. There are mainly three ways available for DNA assembly. The first one involves various integrative sequences, e.g., sites for restriction enzyme, integrase (ligase), and clonase. The second one uses the homologous recombination principle, while the third one is de novo DNA synthesis. Here we will focus mainly on HR-dependent DNA assembler.

*Saccharomyces cerevisiae* has advantage over *E. coli* system for assembly of large fragments of DNA inside the cell. If we are focusing on *E. coli* system for the assembly of DNA fragments, then we have to first clone in a cloning vector with expression cassette, then only we can assemble the sequences. However, the presence of high HR activity of *S. cerevisiae* bypasses these steps, and the assembly of large DNA fragments directly in the chromosomes of yeast is possible. In yeast, it is possible to integrate the in vivo-assembled constructs into the target site in the genome. This can be achieved by designing the 5′ 3 ends of the construct to be homologous and to flanking chromosome sequences of the target insertion, and it must be coupled with selection marker.

#### 7.5.1.2 Genome Editing Tool

The foundation of SB is the pathway assembly for the production of value-added products such as biofuel. Pathway assembly is the basis of synthetic biology for synthesizing value-added products. There exists a strain-dependent variation in the efficiency of various pathways. The efficiency can be increased or improved with the help of SB. For example, the xylose utilization pathway consists of mainly five components, i.e., XYL1, XYL2, XYL3, PHO13, and ALD6. This pathway was inefficient in the strain of *S. cerevisiae*. However, ALD6 and PHO13, two of the genes of this pathway, were mutated on the chromosome by evolutionary engineering as well as optimal expression of all the three XYL genes for generating a highly xylose fermenting strain (Kim et al. 2013). Therefore, for construction of optimized strains, mutations of target genes on chromosomes are necessary. Among various tools available for genome editing, few such as transcription activator-like effector (TALE) nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) and Zinc-finger nucleases (ZFNs) are the best studied and applied.

### 7.5.2 Application of Yeast in Biofuel Production

Yeast (*Saccharomyces cerevisiae*) serves as a chassis in SB due to its small sequenced genome, small life cycle, and easy handling in laboratory. It was only few decades back when engineered yeast was used for the production of biofuels from cellulosic sugars. Since then, tremendous efforts have been made to *utilize* this chassis as efficient model for cost-effective biofuel production. However, there are constrains while using yeast as a model system for this purpose. These challenges are as follows: to broaden the substrate ranges from just sugars to biomass, to develop new synthesis pathways for producing advanced biofuels, and to minimize product inhibition by toxic. So further research has been made to address these problems and solving these problems for biofuel production. To increase the production of biofuels, the substrate utilization was broadened so that substrates other than cellulosic sugars can be utilized.

#### 7.5.2.1 Utilization of Various Substrate for Increased Biofuel Production

It took a very hard work and efforts to expand and increase the substrate range for biofuel production by genetic, metabolic, and protein engineering. The efforts had been made to construct heterologous pathways in yeast for efficient fermentation of non-glucose carbon sources such as galactose, cellobiose arabinose, and xylose. This was possible just because of the discovery of various metabolic enzymes and transporters for utilization of nonglucose sugars. The next step was to improve the expression level of each unit of metabolic pathways via SB such as by utilizing strong and constitutive promoters as well as terminators. Along with this, various cofactors had also been modulated by protein engineering for efficient and broaden substrate utilization for biofuel production. All the necessary and important factors are shown in Table 7.2. With this table, it can be concluded that modulation of the expression of rate-limiting enzymes and optimization of heterologous metabolic pathways are the keys for improving the substrate utilization of sugars other than glucose sugars (Tsai et al. 2015).

#### 7.5.2.2 Advanced Biofuel Production by Engineered Yeast

In the present scenario, ethanol is a biofuel, which can be produced at a huge scale. However, there are few problems associated with the utilization of ethanol as liquid biofuel. So, there was a need for the production of biofuels which is almost parallel to petroleum-based fuel. Metabolic pathways to produce various biofuels such as isobutanol, 1-butanol, isoprenoids, and fatty acid ethyl esters have been reconstituted in yeast (Table 7.3).

**Table 7.3** Various approaches for broadening substrate utilization for biofuel production (Tsai et al. 2015)

| S. no. | Various approaches for broadening substrate utilization | Impact of the strategy |
|---|---|---|
| 1 | Protein engineering of xylose reductase of *Candida tenuis* | Increase in the ethanol yield by 42%<br>Decrease in xylitol by 51% |
| 2 | Overexpression of *Clostridium* phytofermentans XI for codon Optimization | 0.43 g g − 1 ethanol yield<br>0.03 g g − 1 h − 1 sp. ethanol productivity<br>0.07 g g − 1 h − 1 sp. xylose uptake |
| 3 | Overexpressed *Piromyces* xylose isomerase, *S. stipites xylulokinase*, and pentose phosphate pathway | 1.87 g g − 1 h − 1 sp. xylose uptake<br>0.41 g g − 1 ethanol yield (after evolutionary engineering) |

## 7.6 Development in Synthetic Biology Tools for New Drug Discovery

Synthetic biology is redefining the field of drug discovery in the same way as organic chemistry did in pharmaceutical industries a century ago (Trosset and Carbonell 2015). "Since ages nature is used as a source of human medicines, but this process is arduous and very expensive". Often the large-scale production of these compounds being laborious, complex, and non-profitable has led the pharmaceutical industries to abandon these natural medicinal compounds. Being able to biosynthesize these compounds from the source organisms to genetically friendlier hosts would change the scenario. SB is an attempt to apply the concepts of engineering to transform the biological cells into an industrial biofactory. These organisms can be engineered to have either act as biosensors or help us understand the intricacies of living systems or have desirable novel biosynthetic capabilities (Neumann and Neumann-Staubitz 2010).

Initially the application of SB in drug discovery was to enhance the creation of novel chemicals that have properties similar to well-known natural products used for medicinal purposes (Atanasov et al. 2015). The current advances in molecular biology, protein engineering tools, and genome editing are leading a pathway toward the discovery of biological systems that can produce controlled phenotypes using molecular switches. Genetic circuits support the different steps in drug discovery as well as use microorganisms for bioproduction of drugs (Breitling and Takano 2015) in pharmaceutical research.

The advances in the field of DNA technology like inexpensive DNA synthesis, the next-generation DNA sequencing technologies, and well-characterized genetic modules have drawn attention for synthetic biology (Neumann and Neumann-Staubitz 2010). The principles of molecular biology can be applied to reprogram the microorganisms not only to produce the desired drugs but also to deliver it at the

site of action. Here is an overview of how recent development in synthetic biology has revolutionized the field of pharmaceutical biotechnology.

### 7.6.1 Basic Concepts of SB for Drug Development

An archetypal synthetic cell is composed of three basic elements, namely an inducer of gene expression, gene circuit to control the expression of specific genes, and reporter genes to control the output signals related to a disease phenotype (Trosset and Carbonell 2015). To enable the gene expression of targeted compound or for exploration of chemical space of natural products, the gene circuits from enigmatic biosynthetic units of a microorganism can be integrated into host microorganism. Optogenetic biosensors, e.g., lux operon, protein photo sensor like green fluorescent protein can be used to understand drug's mechanism of action through a designed disease model, induce a drug delivery mechanism at a specific site or under specific condition, and validate drug targets. For studying the drug resistance or persistence mechanism or optimize secondary metabolism or fight toxic effects in bacteria, synthetic quorum sensing can be used. Protein engineering is used to modify enzymes or shuffle biosynthetic modules to explore chemical diversity of secondary metabolites (Table 7.4).

### 7.6.2 Mining NPs Space

Biosynthetic machinery of microorganisms, fungi, and plants (Mattern et al. 2015) produces NP derivatives with therapeutic interest. Polyketides (Cummings et al. 2014) non-ribosomal peptides (NRPs), isoprenoids, terpenoids, flavonoids, and alkaloids (Cummings et al. 2014; Trosset and Carbonell 2015) are some of the widely used NPs. The basic idea of using a cell as a biofactory started in early 1900s, when scientist Katz and Leadlay showed that the antibiotic erythromycin was synthesized by a unique gene cluster which forms a giant biosynthetic unit made

**Table 7.4** Synthetic biology tools in various steps of drug discovery

| Synthetic biology | Drug discovery |
|---|---|
| Genetic circuits in host organisms | Increase flux of secondary metabolic pathways |
| Protein engineering—modify enzymes or shuffles biosynthetic modules | Explore chemical diversity of secondary metabolites |
| Optogenetics biosensing | Target validation<br>Drug mechanism of action<br>Disease models<br>Drug discovery |
| Synthetic quorum sensing: cell–cell communication | Overcome drug resistance<br>Optimize secondary metabolites<br>Fight toxic effects |

of 28 protein modules (Donadio et al. 1991; Walsh and Fischbach 2010). These biosynthetic units could be further isolated and applied to modify at the genetic level to produce NP derivatives into host organism (Newman and Cragg 2012). Bioinformatics tools, such as antibiotics and Secondary Metabolite Analysis Shell (antiSMASH) and Secondary Metabolite Unknown Regions Finder (SMURF) (Wohlleben et al. 2012; Doroghazi et al. 2014), which are used for large-scale genome or metagenomes sequencing of microorganisms have extended the discovery of such biosynthetic gene clusters (Cacho et al. 2015). Even though most of the biosynthetic gene clusters remain cryptic or silent under usual conditions, lots of effort have been made to boost or reactivate silent gene expression through the design of ligand-controlled aptamers, synthetic transcription factors (TFs) (Leavitt and Alper 2015), or riboswitches (Shao et al. 2013) "knock-in" promoter replacement strategy (Harvey et al. 2015).

### 7.6.3 In Vivo Combinatorial Libraries

Combinatorial libraries are widely used in pharmaceutical industries for drug discovery. However, owing to perceived failures with this technology, different in vivo compound libraries can be produced for the drug discovery. Such libraries would have several advantages like maintenance, and amplification would be simply done by cultivating cells. Since, genetic information of the cell is directly linked to the compound, it can be used to screen the compound. Thirdly, limitations of in vitro assays for enzymatic activity can be overcome by an intracellular genetic selection. Also, greater level of sensitivity of the drug for its target could be achieved when the selection is performed in context to the living cell. Finally, problems with uptake and solubility of the compounds could be avoided.

Antibiotics like valinomycin and fungal toxins like amatoxins, phalloidin are few of the natural compounds which are modified cyclic peptides providing resistance to cellular degradation and restricts conformational freedom, thus potentially improving binding affinity and specificity (Katsara et al. 2006). Polyketides are another important class of natural products prominently produced by actinomycetes, (e.g., erythromycin, anthracyclines, indolocarbazoles, isoprenoids, and epothilone). Their individual modules have been split and recombined to form active enzymes which catalyzed the formation of polyketides and their precursors like epothilone C and D (anticancer drugs), aklanoic acid (which is a precursor to several antitumor polyketides like aclacinomycin A and doxorubicin) in *E. coli*. This approach thus can be used to develop a potential compound with novel biological activity.

### 7.6.4 Metabolic Engineering for Drug Production

The most significant and well-known use of SB is for the production of artemisinin, which is an antimalarial drug, traditionally made from materials naturally produced by the sweet wormwood tree. Scientists at Amyris altered the genome of yeast,

adding six genes that enabled it to produce artemisinic acid, a precursor component that can be converted into the medication thus generating this therapeutic faster and cheaper than its natural source. Similar approach has been used for the production of isoprenoid paclitaxel (also known as taxol), a drug used for chemotherapy. As its chemical synthesis and natural extraction from Pacific yew is difficult, and inefficient modular approach was used for its production, achieving a titer of 1 g/L in *E. coli*.

## 7.7 Conclusion

In this chapter, many of the thought-provoking applications from the area of synthetic biology are discussed. In most of the cases, these are the representatives of a handful of strains with relatively simple interactions. However, these advances have future application using the new emerging tools in the field of synthetic biology, molecular biotechnology, and mathematical modeling. Upcoming advances in the area of synthetic biology have the potential to renovate fields of bioproduction, medicine, environmental engineering, and bioprocessing. Synthetic biology has a vast application in the field and has a potential to solve various problems in the field of biotechnology via mathematical modeling as well as applying the engineering in biotechnology. Additionally in future we also have to check the integration and response of the synthetically designed strain on the pressure of the evolution whether these strains are stable enough during the course of evolution. Recently emerging bioinformatics and computational tools have shown a futuristic word with modified organisms with enhanced value-added product as well as engineered strains. However, in reality these need to be further checked.

## References

Allen RS, Tilbrook K, Warden AC, Campbell PC, Rolland V, Singh SP, Wood CC (2017) Expression of 16 Nitrogenase proteins within the plant mitochondrial matrix. Front Plant Sci 8:1–14

Andrianantoandro E, Basu S, Karig DK, Weiss R (2006) Synthetic biology: new engineering rules for an emerging discipline. Mol Syst Biol 2(1):2006–2028

Atanasov AG, Waltenberger B, Pferschy-Wenzig E-M et al (2015) Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 15:S0734–S9750

Awan AR, Shaw WM, Ellis T (2016) Biosynthesis of therapeutic natural products using synthetic biology. Adv Drug Deliv Rev 105:96–106

Belkin S (2003) Microbial whole-cell sensing systems of environmental pollutants. Curr Opin Microbiol 6:206–212

Breitling R, Takano E (2015) Synthetic biology advances for pharmaceutical production. Curr Opin Biotechnol 35C:46–51

Cacho RA, Tang Y, Chooi Y-H (2015) Next-generation sequencing approach for connecting secondary metabolites to biosynthetic gene clusters in fungi. Front Microbiol 5(774):1–16

Carpenter A, Paulsen I, Williams T (2018) Blueprints for biosensors: design, limitations, and applications. Genes 9(8):375

Chou HH, Keasling JD (2013) Programming adaptive control to evolve increased metabolite production. Nat Commun 4:2595
Cravens A, Payne J, Smolke CD (2019) Synthetic biology strategies for microbial biosynthesis of plant natural products. Nat Commun 10(1):2142
Cummings M, Breitling R, Takano E (2014) Steps towards the synthetic biology of polyketide biosynthesis. FEMS Microbiol Lett 351(2):116–125
D'Andrea L, Simon-Moya M, Llorente B, Llamas E, Marro M, Loza-Alvarez P, Li L, Rodriguez-Concepcion M (2018) Interference with Clp protease impairs carotenoid accumulation during tomato fruit ripening. J Exp Bot 69:1557–1567
Dietrich JA, Shis DL, Alikhani A, Keasling JD (2013) Transcription factor-based screens and synthetic selections for microbial small-molecule biosynthesis. ACS Synth Biol 2:47–58
Donadio S, Staver MJ, McAlpine JB, Swanson SJ, Katz L (1991) Modular organization of genes required for complex polyketide biosynthesis. Science 252(5006):675–679
Doroghazi JR, Albright JC, Goering AW et al (2014) A roadmap for natural product discovery based on large-scale genomics and metabolomics. Nat Chem Biol 10(11):963–968
Findeiss S, Etzel M, Will S, Morl M, Stadler PF (2017) Design of artificial riboswitches as biosensors. Sensors 17:1990
Galanie S, Thodey K, Trenchard IJ, FilsingerInterrante M, Smolke CD (2015) Complete biosynthesis of opioids in yeast. Science 349:1095–1100
Giessen TW, Silver PA (2017) Engineering carbon fixation with artificial protein organelles. Curr Opin Biotechnol 46:42–50
Goold HD, Nguyen HM, Kong F, Beyly-Adriano A, Légeret B, Billon E, Cuiné S, Beisson F, Peltier G, Li-Beisson Y (2016) Whole genome re-sequencing identifies a quantitative trait locus repressing carbon reserve accumulation during optimal growth in Chlamydomonas reinhardtii. Sci Rep 6:25209
Harvey AL, Edrada-Ebel R, Quinn RJ (2015) The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 14(2):111–129
Hill MK (2004) Understanding environmental pollution. A primer. Cambridge University Press, Cambridge
Katsara M, Tselios T, Deraos S, Deraos G, Matsoukas MT, Lazoura E, Matsoukas J, Apostolopoulos V (2006) Round and round we go: cyclic peptides in disease. Curr Med Chem 13:2221–2232
Kim SR, Skerker JM, Kang W, Lesmana A, Wei N, Arkin AP, Jin YS (2013) Rational and evolutionary engineering approaches uncover a small set of genetic changes efficient for rapidxylose fermentation in Saccharomyces cerevisiae. PLoS One 8:e57048
Leavitt JM, Alper HS (2015) Advances and current limitations in transcript-level control of gene expression. Curr Opin Biotechnol. 34(3):98–104
Leavitt JM, Wagner JM, Tu CC, Tong A, Liu Y, Alper HS (2017) Biosensor-enabled directed evolution toimprovemuconic acid production in Saccharomyces cerevisiae. Biotechnol J 12:1600687
Lee YJ, Hoynes-O'Connor A, Leong MC, Moon TS (2016) Programmable control of bacterial gene expression with the combined CRISPR and antisense RNA system. Nucleic Acids Res 44 (5):2462–2473
Leonard P, Hearty S, Brennan J, Dunne L, Quinn J, Chakraborty T, O'Kennedy R (2003) Advances in biosensors for detection of pathogens in food and water. Enzyme Microbial Technol 32:3–13
Liu W, Luo Z, Wang Y, Pham NT, Tuck L, Pérez-Pi I, Liu L, Shen Y, French C, Auer M et al (2018) Rapid pathway prototyping and engineering using in vitro and in vivo synthetic genome SCRaMbLE-in methods. Nat Commun 9:1936
Llorente B, Martinez-Garcia JF, Stange C, Rodriguez-Concepcion M (2017) Illuminating colors: regulation of carotenoid biosynthesis and accumulation by light. Curr Opin Plant Biol 37:49–55
Mattern DJ, Valiante V, Unkles SE, Brakhage AA (2015) Synthetic biology of fungal natural products. Front Microbiol 6:775

McKeague M, Velu R, Hill K, Bardóczy V, Mészáros T, DeRosa M (2014) Selection and characterization ofa novel DNA aptamer for label-free fluorescence biosensing of Ochratoxin A. Toxins 6:2435

Muranaka N, Sharma V, Nomura Y, Yokobayashi Y (2009) Efficient design strategy for whole-cell and cell-free biosensors based on engineered riboswitches. Anal Lett 42:108–122

Nagai T, Sawano A, Park ES, Miyawaki A (2001) Circularly permuted green fluorescent proteins engineered to sense Ca2+. Proc Natl Acad Sci U S A 98:3197–3202

Neumann H, Neumann-Staubitz P (2010) Synthetic biology approaches in drug discovery and pharmaceutical biotechnology. Appl Microbiol Biotechnol 87(1):75–86

Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75(3):311–335

Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R, Croce R, Hanson MR, Hibberd JM, Long SP et al (2015) Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc Natl Acad Sci U S A 112:8529–8536

Petrie JR, Shrestha P, Zhou XR, Mansour MP, Liu Q, Belide S, Nichols PD, Singh SP (2012) Metabolic engineering plant seeds with fish oil-like levels of DHA. PLoS One 7:e49165

Puchta H (2016) Using CRISPR/Cas in three dimensions: towards synthetic plant genomes, transcriptomes and epigenomes. Plant J 87:5–15

Ravikumar S, Baylon MG, Park SJ, Choi J i (2017) Engineered microbial biosensors based on bacterial two-component systems as synthetic biotechnology platforms in bioremediation and biorefinery. Microb Cell Fact 16:1–10

Rogers C, Oldroyd GED (2014) Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. J Exp Bot 65:1939–1946

Shao Z, Rao G, Li C, Abil Z, Luo Y, Zhao H (2013) Refactoring the silent spectinabilin gene cluster using a plug and play scaffold. ACS Synth Biol 2(11):662–669

Shri M, Dave R, Diwedi S, Shukla D, Kesari R, Tripathi RD, Trivedi PK, Chakrabarty D (2014) Heterologous expression of Ceratophyllum demersum phytochelatin synthase, CdPCS1, in rice leads to lower arsenic accumulation in grain. Sci Rep 4:5784

Siedler S, Schendzielorz G, Binder S, Eggeling L, Bringer S, Bott M (2014) SoxR as a single-cell biosensor for NADPH consuming enzymes in Escherichia coli. ACS Synth Biol 3:41–47

Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J (2015) Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci 16(10):24673–24706

Song S, Wang L, Li J, Fan C, Zhao J (2008) Aptamer-based biosensors. TrAC Trends Anal Chem 27:108–117

Stein V, Alexandrov K (2015) Synthetic protein switches: design principles and applications. Trends Biotechnol 33:101–110

Steindler L, Venturi V (2007) Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors. FEMS Microbiol Lett 266(1):1–9

Trosset JY, Carbonell P (2015) Synthetic biology for pharmaceutical drug discovery. Drug Des Devel Ther 9:6285

Tsai CS, Kwak S, Turner TL, Jin YS (2015) Yeast synthetic biology toolbox and applications for biofuel production. FEMS Yeast Res 15(1):1–15. https://doi.org/10.1111/1567-1364.12206

Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249:505–510

Walsh CT, Fischbach MA (2010) Natural products version 2.0: connecting genes to molecules. J Am Chem Soc 132(8):2469–2493

Wan X, Volpetti F, Petrova E, French C, Maerkl SJ, Wang B (2019) Cascaded amplifying circuits enable ultrasensitive cellular sensors for toxic metals. Nat Chem Biol 15(5):540

Wei Y, Bergenholm D, Gossing M, Siewers V, Nielsen J (2018) Expression of cocoa genes in Saccharomyces cerevisiae improves cocoa butter production. Microb Cell Factories 17:11

Wen KY, Cameron L, Chappell J, Jensen K, Bell DJ, Kelwick R, Kopniczky M, Davies JC, Filloux A, Freemont PS (2017) A cell-free biosensor for detecting quorum sensing molecules in P. aeruginosa-infected respiratory samples. ACS Synth Biol 6(12):2293–2301

Williams TC, Xu X, Ostrowski M, Pretorius IS, Paulsen IT (2017) Positive-feedback, ratiometric biosensor expression improves high-throughput metabolite-producer screening efficiency in yeast. Synth Biol 2:ysw002

Wohlleben W, Mast Y, Muth G, Röttgen M, Stegmann E, Weber T (2012) Synthetic biology of secondary metabolite biosynthesis in actinomycetes: engineering precursor supply as a way to optimize antibiotic production. FEBS Lett 586(15):2171–2176

Yagi K (2007) Applications of whole-cell bacterial sensors in biotechnology and environmental science. Appl Microbiol Biotechnol 73:1251–1258

Zhang F, Carothers JM, Keasling JD (2012) Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids. Nat Biotechnol 30:354