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Minireview

Applications and advances of metabolite biosensors for metabolic engineering

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ABSTRACT

Quantification and regulation of pathway metabolites is crucial for optimization of microbial production bioprocesses. Genetically encoded biosensors provide the means to couple metabolite sensing to several outputs invaluable for metabolic engineering. These include semi-quantification of metabolite concentrations to screen or select strains with desirable metabolite characteristics, and construction of dynamic metabolite-regulated pathways to enhance production. Taking inspiration from naturally occurring systems, biosensor functions are based on highly diverse mechanisms including metabolite responsive transcription factors, two component systems, cellular stress responses, regulatory RNAs, and protein activities. We review recent developments in biosensors in each of these mechanistic classes, with considerations towards how these sensors are engineered, how new sensing mechanisms have led to improved function, and the advantages and disadvantages of each of these sensing mechanisms in relevant applications. We particularly highlight recent examples directly using biosensors to improve microbial production, and the great potential for biosensors to further inform metabolic engineering practices.

1. Introduction

Advances in metabolic engineering have enabled microbial production of a wide variety of valuable compounds, providing alternative synthesis routes for chemicals including biofuels, pharmaceuticals, nutraceuticals, bulk chemicals, and materials. To produce these valuable compounds, efficient biosynthetic pathways must be constructed in appropriate hosts, which often requires extensive optimization to reach economically viable titers, yields, and productivities. The cycle of repeatedly tuning pathway parameters and evaluating production is laborious and time-consuming. Synthetic biology is a fast-growing field that develops new tools for biological engineering, fulfilling the need for efficient pathway optimization. It has proven effective in increasing process predictability and throughput as well as in creating new strategies to optimize biosynthetic pathways. Among these new tools, biosensors represent a significant contribution from synthetic biology and have been increasingly used in metabolic engineering. Here, we review recent work on the development of metabolite biosensors and their applications for metabolic engineering.

Biosensors are ubiquitous in nature and have evolved to detect both environmental signals (e.g. temperature, pH, oxygen) and intra- and extracellular metabolites. These sensed signals are coupled with actuator outputs to modify the transcription, translation, and protein activities of cells. A crucial consideration in synthetic biology and metabolic engineering is that these natural biosensing machineries have evolved to maximize evolutionary fitness rather than overproduction of any metabolite. However, detailed knowledge of the sensing mechanisms and endogenous functions of these biosensors serve as a valuable starting point to co-opt them for overproduction goals.

This review will focus on "metabolite biosensors" developed for metabolic engineering applications. We define "metabolite biosensors" as genetically-encoded protein or RNA-based sensors that interact with a metabolite to generate an actuator output. The output domain of a metabolite biosensor generates detectable phenotypes through modulating transcription rates, translation rates, or post-translational parameters to control protein expression or activity. Over the past few decades, metabolite biosensors have drawn tremendous attention and have several applications in metabolic engineering (Fig. 1). *First*, biosensors can be coupled to readable outputs such as fluorescence to semi-quantitatively report the concentration of a

Abbreviations: MRTF, metabolite-responsive transcription factor; MAGE, multiplex automated genome engineering; FAEE, fatty acid ethyl ester; ACC, acetyl-CoA carboxylase; TCS, two-component system; HK, histidine kinase; RR, response regulator; FPP, farnesyl pyrophosphate; FRET, forster resonance energy transfer; FPX, fluorescent protein exchange; PBP, periplasmic binding protein; IPP, isopentenyl diphosphate; MCFA, medium-chain fatty acid

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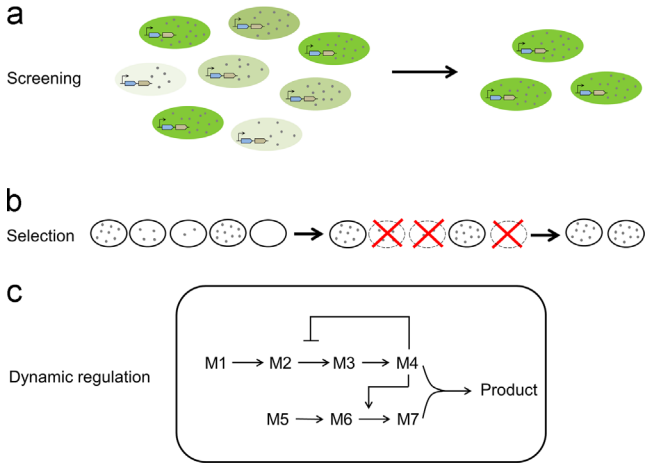

Fig. 1. Applications of metabolite biosensors in metabolic engineering: (a) biosensors can be linked to a colorimetric output to report the concentration of metabolites, providing a straightforward way to screen for high-producing strains. (b) Biosensors can be used to control an output associated with a fitness advantage under selective conditions. This allows direct enrichment and selection of high-producers. (c) Biosensors can be used to control the activity of a metabolic pathway, which allows dynamic optimization of the pathway activity according to the level of the sensed metabolite.

target compound. This approach is frequently used for high-throughput screening of high-producing strains and features distinct advantages over conventional methods such as gas chromatography (GC) and high-performance liquid chromatography (HPLC): (1) biosensor-mediated quantification avoids time-consuming sample preparation and has much higher throughput than conventional chromatographic techniques; (2) metabolite biosensors are more suitable for detecting labile and low abundant metabolites such as acyl-phosphate, acyl-diphosphate, aldehyde, and acyl-CoAs, which are difficult to measure accurately by conventional methods; (3) metabolite biosensors allow real-time monitoring of metabolite dynamics in living cells, which is impossible to study using chromatographic methods. These reporter outputs may also help coordinate complementary manipulations of the culture environment itself (mixing, nutrient addition, timing of harvest) to further improve production (Polizzi and Kontoravdi, 2015). *Second*, biosensors can be engineered to couple the sensing of a desirable product or intermediate metabolite with a fitness advantage for the cell by expressing a gene necessary for survival under selective conditions (Dietrich et al., 2013; Raman et al., 2014). The difference in cell growth allows direct enrichment of fast-growing cells from mutant libraries, which allows an easy selection for desirable production characteristics. *Third*, metabolite biosensors can also be used to control metabolic flux dynamically (Dahl et al., 2013; Liu et al., 2013; Zhang et al., 2012; Zhou and Zeng, 2015). The actuator can be designed to tune pathway enzyme expression or post-translational parameters in response to the level of the relevant metabolite, allowing for dynamic control of pathway activity based on the cellular metabolic state. As a result, the pathway is dynamically balanced, which not only reduces toxic intermediate accumulation but also saves carbon and energy that is otherwise diverted to synthesize unnecessary proteins or intermediates. Overall, the emerging tools to engineer biosensors and their applications towards metabolic engineering have greatly advanced microbial production of a variety of chemicals.

While biosensors have been reviewed previously (Gredell et al., 2012; Michener et al., 2012; Palmer et al., 2011; Schallmey et al., 2014; Su et al., 2011; Zhang and Keasling, 2011), this review focuses on recent advances in metabolite biosensors and emphasizes their applications for metabolic engineering. Here we classify metabolite biosensors into five categories based on their diverse mechanisms of sensing and functional output, including (1) metabolite-responsive transcription factors, (2) two-component systems, (3) cellular stress response, (4) regulatory RNAs, and (5) protein activities. Biosensors that detect environmental signals have been previously reviewed (Salis et al., 2009; Van Dorst et al., 2010; Zhang and Keasling, 2011) and will not be discussed here.

2. Metabolite biosensors

2.1. Biosensors based on metabolite-responsive transcription factors

In nature, transcription factors regulate gene expression by specific binding to the chromosomal DNA, blocking or promoting transcription by RNA polymerase. Among these, some transcription factors can be activated or deactivated by small molecules through ligand binding, phosphorylation, or interaction with other regulatory elements. Here we will focus on transcription factors that respond to metabolites.

Metabolite-responsive transcription factors (MRTFs) have evolved to interact with various metabolites. *Escherichia coli*, for example, has more than 230 transcription factors (Binder et al., 2012), which sense a wide variety of metabolites, including sugars, sugar phosphates, amino acids, and lipids. Natural MRTFs have been extensively explored to engineer biosensors for metabolic engineering applications. Typically, metabolite-responsive promoters with tunable output dynamic ranges can be engineered by inserting the cognate operator of a MRTF into a synthetic promoter to regulate genes of interest (Fig. 2a and b). Using this strategy, biosensors that respond to a variety of metabolites have been created, including sensors for butanol (Dietrich et al., 2013), alkanes (Reed et al., 2012), malonyl-CoA (Liu et al., 2013; Xu et al., 2014), acyl-CoA (Zhang et al., 2012) and aromatic aldehyde (Fiorentino et al., 2009). The primary use of MRTF sensors is to screen for high-producing strains from a library of natural or engineered strains, as demonstrated in the production of several chemicals, including mevalonate (Tang and Cirino, 2011), L-lysine (Binder et al., 2012), and triacetic acid lactone (Tang et al., 2013). This approach becomes particularly powerful when coupled with fluorescence-activated cell sorting (FACS). In one example, an *eyfp* was cloned 3′ of a *Corynebacterium glutamicum* promoter that is regulated by an endogenous transcription factor Lrp, which can detect L-methionine and several branched-chain amino acids, including L-valine, L-leucine and L-isoleucine (Mustafi et al., 2012). Using chemical mutagens, random mutations were introduced to the *C. glutamicum* strains, which carry the sensor plasmid. Cells were cultivated and screened by FACS, and the ones with enhanced fluorescence were isolated and re-cultivated to enrich the high-producing strains. Mutants that produce up to a total of 11 mM branched-chain amino acids were identified. In addition, MRTFs have also been used to control genes associated with cell growth/survival for selection (Dietrich et al., 2013; Raman et al., 2014). In a recent paper, Raman et al. used MRTF-regulated promoters to control the expression of TolC, a protein that allows both positive and negative selections when supplemented with sodium dodecyl-sulfate (SDS) and colicin E1, respectively. While positive selections were needed to select for high-producing strains generated by multiplex automated genome engineering (MAGE), negative selections were used to eliminate the false positives caused by mutations that deactivate the sensor-selection system. By alternating between negative and positive cycles, the authors demonstrated enhanced production for both naringenin and glucaric acid (Raman et al., 2014). Overall, biosensor-mediated high-throughput screening and selection methods drastically shorten the time required to analyze mutant cells, enhancing the power of evolutionary approaches. In addition, MRTF-based biosensors have also been used to dynamically regulate metabolic flux. One of the earliest examples of using the MRTF sensor for dynamic regulation involves a fatty acyl-CoA biosensor FadR (Zhang et al., 2012). FadR naturally regulates several genes in *E. coli* fatty acid