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Research review paper

Synthetic biology: Tools to design microbes for the production of chemicals and fuels

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ABSTRACT

The engineering of biological systems to achieve specific purposes requires design tools that function in a predictable and quantitative manner. Recent advances in the field of synthetic biology, particularly in the programmable control of gene expression at multiple levels of regulation, have increased our ability to efficiently design and optimize biological systems to perform designed tasks. Furthermore, implementation of these designs in biological systems highlights the potential of using these tools to build microbial cell factories for the production of chemicals and fuels. In this paper, we review current developments in the design of tools for controlling gene expression at transcriptional, post-transcriptional and post-translational levels, and consider potential applications of these tools.

Contents

1. Introduction

Interest in bio-based production of chemicals and fuels as an alternative industrial synthesis route has steadily grown owing to the finite nature of fossil resources and environmental concerns (Ganesh et al., 2012; Jang et al., 2012; Zhang et al., 2012b). Evidence of this interest is found in recent developments in the field of synthetic biology, which has promoted numerous breakthrough technologies for redesigning existing biological systems and synthesizing new ones to deal with these problems (Lynch and Gill, 2012; Seo et al., 2012; Xing et al., 2012). Building a synthetic biological system requires optimization of a number of components, including genetic parts, devices and systems, using design tools capable of regulating these components in a predictable and quantitatively controllable manner (Yadav et al., 2012; Zhu et al., 2012). Imbalances within systems can lead to failure to perform the designed program; thus, recent research trends in this field have focused on the development of novel tools that can be utilized to design and optimize biological systems.

Because balanced expression of multiple enzymes that constitute a metabolic pathway or genetic program is a prerequisite for achieving optimal performance of the designed biological system, controlling gene expression in a predictable and quantitatively controllable manner is one of the most important design principles. Gene expression can be regulated at transcriptional, post-transcriptional, and post-translational levels. In this paper, we review recent developments in the design of

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tools for predicting and quantitatively controlling gene expression at all three levels. We also gauge their applicability for the production of chemicals and fuels, and discuss future perspectives in this area.

2. Design tools for controlling gene expression at the transcriptional level

Transcriptional control of gene expression has been widely used in metabolic engineering and synthetic biology applications to optimize biological systems such as metabolic pathways and genetic circuits. One such approach is to create synthetic promoter libraries with a broad range of transcription efficiencies in a variety of prokaryotes (Alper et al., 2005; Braatsch et al., 2008; Miksch et al., 2005; Rud et al., 2006) and eukaryotes (Alper et al., 2006; Qin et al., 2011). Conventional approaches involve modification of the spacer region between the consensus hexameric DNA sequences (−10 and −35 boxes) of native promoters or utilization of error-prone polymerase chain reaction (PCR) to introduce mutations into the entire promoter region (Fig. 1A). Recently, several studies have utilized synthetic hybrid promoter approaches that combine core promoters with enhancer elements consisting of combinations or tandem repeats of upstream activating sequences (UAS elements) in yeasts (Blazeck et al., 2011, 2012). By expanding the promoter architecture, this approach could further enhance promoter strength, control the regulation mechanism, and consequently establish a library spanning a broad dynamic range of expression levels.

Because promoter strength can differ depending on flanking sequences upstream and downstream of the consensus boxes as well as promoter copy number (Davis et al., 2011), it is necessary to standardize ways to ensure that the expected transcription efficiency can be achieved when using previously developed promoter sequences as a modular part in either chromosomes or plasmids (Kelly et al., 2009). A recent study demonstrated the importance of precisely balancing metabolic fluxes by controlling transcription efficiency for the optimal production of a target molecule (Fig. 1B) (Ajikumar et al., 2010). Using multivariate-modular systems harboring different promoter strengths and copy numbers, Ajikumar et al. (2010) successfully optimized the isoprenoid pathway for the production of taxadiene, a paclitaxel precursor. In this application, the taxadiene biosynthetic pathway was divided into two modules regulated by separate promoters with different strengths and copy numbers. By employing a systematic approach, they dramatically improved titers up to 15,000-fold in the final strain. This study utilized a limited number of promoters and a plasmid-based expression system. However, the increasing number of synthetic promoters with a broad dynamic range and the availability of technology for easily duplicating genes in chromosomes will facilitate the search of a larger solution-space and aid in the development of long-term genetically stable strains (Blazeck and Alper, 2012; Tyo et al., 2009).

Eliciting complex cellular phenotypes such as tolerance to chemicals and fuels requires reprogramming gene networks and metabolism because such traits are generally regulated by multiple genes. One strategy for accomplishing this is global transcription machinery engineering (gTME), which has recently been implemented in both prokaryotes (e.g., *Escherichia coli*, Alper and Stephanopoulos, 2007; *Lactobacillus plantarum*, Klein-Marcuschamer and Stephanopoulos, 2008) and eukaryotes (*Saccharomyces cerevisiae*, Alper et al., 2006; Liu et al., 2010) (Fig. 1C). In theory, this approach creates libraries of mutant transcription factors, which in turn affect the expression of multiple genes and lead to reprogramming of gene networks. With appropriate phenotype selection and screening, reprogrammed networks that elicit the desired phenotype can be identified and characterized. However, the connection between mutant transcription factor sequences and function is difficult to predict de novo. Recent advances in understanding DNA-binding sites of transcription factors should facilitate the discovery of structure–function relationships and thus enable the rational, predictable reprogramming of gene networks for strain improvement (Barrett et al., 2011; Cho et al., 2009; Kim et al., 2012).

A recent study by Zhang et al. (2012a,b) reported the development of a dynamic sensor-regulator system (DSRS) that uses a naturally occurring transcription factor/synthetic hybrid promoter pair (Fig. 1D) (Zhang et al., 2012a). This DSRS could sense a key metabolite in the fatty acid production pathway and dynamically regulate the expression of genes involved in fatty acid ethyl ester (FAEE) production.

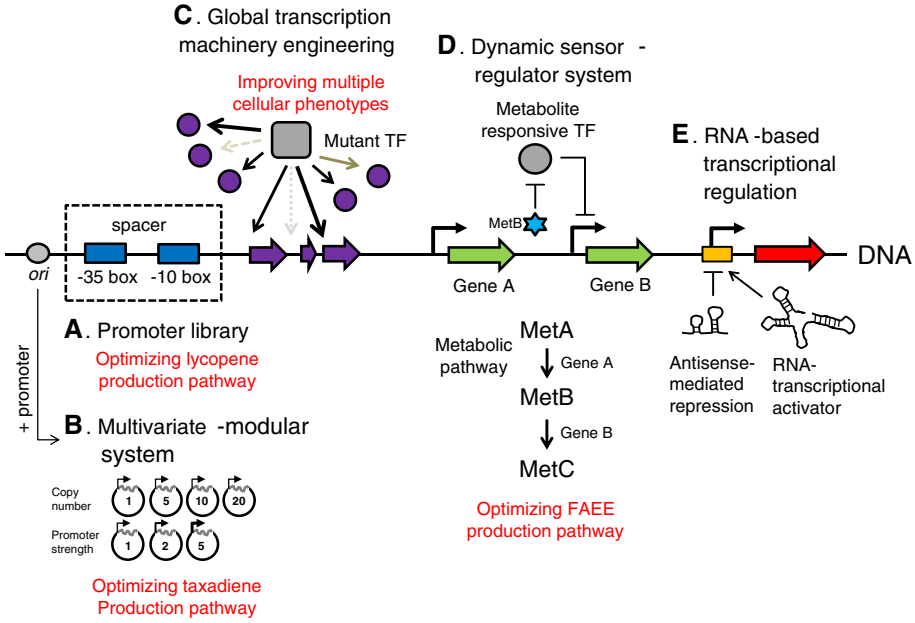

Fig. 1. Design tools for controlling gene expression at the transcriptional level. (A) A promoter library can be constructed by modifying consensus (−35 and −10 boxes) and spacer regions. (B) Transcription can be controlled using a multivariate-modular system containing promoters with various strengths and copy numbers. (C) Transcription machinery elements, such as transcription factor (TFs), can be engineered to perturb the global transcription network. (D) The expression of genes encoding enzymes in a metabolic pathway can be dynamically controlled by metabolite responsive TFs. (E) RNA can control transcription events by acting as a transcriptional activator or repressor through an antisense-mediated mechanism. Examples on application for the production of fuels and chemicals were described in red letters.