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Review

Recent advancements in synthetic biology: Current status and challenges

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ABSTRACT

Synthetic biology is the design and construction of new biological parts, devices and circuits not existing in nature. It provides a novel solution to imminent challenges in a wide variety of fields, including the discovery of new drugs, production chemicals, renewable biofuels, value-added products and cellular reprogramming. Many efforts have been made to design and characterize synthetic genetic parts, including promoter, transcription factors, RBS, degradation tags and transcriptional terminators, among others. These genetic parts have been assembled for construction of a number of synthetic devices and circuits like oscillators, toggle switches, amplifiers and biologic gates; they play a vital role in cell reprogramming for better understanding of cellular mechanisms and control of biological process. They are also useful for the periodic and tunable production of drugs, fine chemicals, vaccines and much more. It is the goal of this review to aid and accelerate future research in synthetic biology.

Contents

Abbreviations: AHL, acyl-homoserine lactone; ATc, anhydrotetracycline; AU, arbitrary units; C4-HSL, *N*-butyryl-homoserine lactone; CAP, catabolite activator protein; CRP, cAMP-receptor protein; HTH, helix-turn-helix; IPTG, isopropyl β-D-1-thiogalactopyranoside; PFLs, positive feedback loops; RBS, ribosomal binding site; RNAP, RNA polymerase; TF, transcription factor; TSS, transcription start site; yemGFP, monomeric yeast-enhanced green fluorescent protein; σ factor, sigma factor.

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1. Introduction

Synthetic biology is defined as "the design and construction of new biological parts, devices, and systems, or the re-design of existing, natural biological systems for useful purposes." It is a new field of biological science in which many disciplines such as physics, chemistry, mathematics, engineering and computer sciences are applied simultaneously. It will play a vital role in the improving and establishing of synthetic gene networks and biosynthetic pathway for better understanding of cellular mechanisms and to provide valuable products for disease prevention.

American and European scientists have designed a number of synthetic gene circuits using synthetic genetic parts with well defined functions. In recent years, research groups around the world have used synthetic biology to better understand diseases as well as food and energy production. It is a rational design of synthetic gene circuits using modularized, standardized parts, which are DNA traits with well-defined functions (Endy, 2005; Marchisio and Stelling, 2011). The first synthetic genetic devices – "Repressilator" (Elowitz and Leibier, 2000) and "Toggle switch" (Gardner et al., 2000) – were successfully constructed and characterized in *Escherichia coli*. Subsequently, a number of synthetic parts including promoters (Alper et al., 2005; Baron et al., 1997; Lutz and Bujard, 1997), regulatory proteins and RNAs (Bayer and Smolke, 2005; Dueber et al., 2003; Isaacs et al., 2004; Pfleger et al., 2006), and scaffolds (Dueber et al., 2009; Park et al., 2003), have been successfully engineered and characterized in a number of hosts.

Synthetic parts have been assembled to construct novel genetic devices and circuits such as oscillators (Danino et al., 2010; Elowitz and Leibier, 2000; Stricker et al., 2008), riboregulators (Isaacs et al., 2004; Lou et al., 2012), riboswitches (Blount and Breaker, 2006; Tucker and Breaker, 2005; Winkler et al., 2002) and biologic gates (Bonnet et al., 2013; Moon et al., 2012; Tamsir et al., 2011). Friedland et al. (2009) constructed and characterized digital circuits that can program and design cells using the principles of modern computing — such as counting. These devices count various user-defined inputs with a range of frequencies that can be expanded to count higher numbers. In the present review, the recently engineered and characterized genetic parts, devices and circuits are highlighted – especially those in *E. coli* – for a better understanding of the cellular mechanisms and opportunity and challenges in the future.

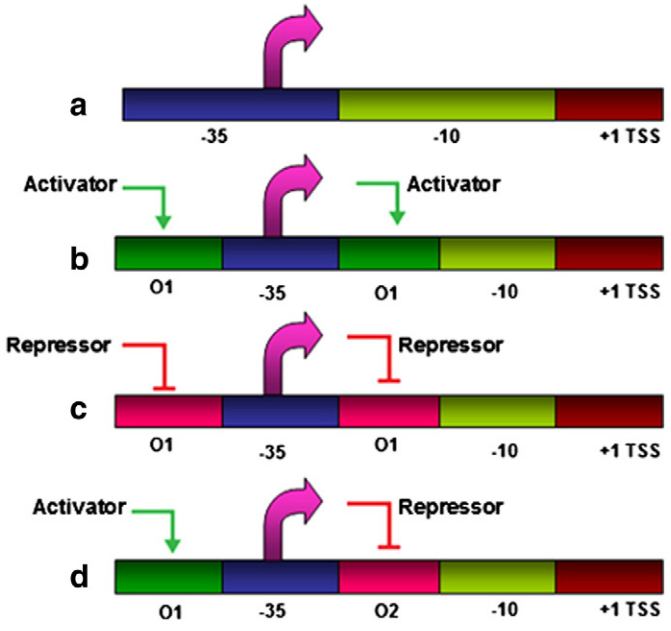

Fig. 1. Schematic representation of different elements present (−35, −10, +1 TSS, Operator site) in *Escherichia coli* based synthetic promoter. (**a**) constitutive, (**b**) inducible, (**c**) repressible and (**d**) combinatorial promoter.

2. Design, construction and characterization of genetic parts

In a recent study Canton et al. (2008), suggested that the ability to quickly and reliably engineer many-component systems from libraries of standard interchangeable parts, is a hallmark of modern technologies. There is a requirement to refine, standardize and modularize the biological parts, devices and circuits.

2.1. Design and modulation of promoter

A promoter is a specific DNA sequence that recruits the transcriptional machinery and facilitates the transcription of a desired gene. The specific sequence of promoters determines their strength by high or low binding with RNA polymerase (RNAP). The major components of *E. coli* promoter sequences are −35 and −10 (Pribnow box), region and operator for repressor or activator or both proteins binding site that can tune the promoter strength. RNAP is associated with the sigma factor for binding at specific regions of the promoter (−35 and −10). When the σ factor and RNAP are combined, a holoenzyme is formed. The σ factor is a protein required for initiation of transcription (the 'transcription initiation factor'), that enables specific binding of RNAP with the promoter. Each molecule of RNAP contains exactly one σ factor subunit, which is dependent on the gene and on the environmental signals (Gruber and Gross, 2003; Sharma and Chatterji, 2010).

There is no operator site which presents a constitutive promoter. It means that there is no effect of the transcription factor (TFs). The promoter contains −35, −10 and the transcription start site (+1 TSS) thus; it can constitutively express the protein (Fig. 1a). An inducible promoter has one or two operator sites for same TF; it binds onto the operator which is induced by the inducer (Fig. 1b). As shown in Fig. 1c, this repressible promoter is repressed by same, or two different TFs, and is activated by inducer molecules. The combined promoter has at least two operator sites for different repressors, which can be activated by using both inducers (Fig. 1d). A number of synthetic promoters could be designed by the promoter engineering to fine-tune gene expressions and help control gene networks.

2.1.1. Constitutive promoter

The unregulated constitutive promoter allows for continual transcription of the desired gene. Liang et al. (1999), identified the seven constitutive promoters in *E. coli*; they include (i), the spc ribosomal protein operon promoter Pspc (ii), the beta-lactamase gene promoter Pbla of plasmid pBR322 (iii), the PL promoter of phage lambda (iv), and (v), the replication control promoters PRNAI and PRNAII of plasmid pBR322 and (vi) and (vii), the P1 and P2 promoters of the rrnB ribosomal RNA operons.

Initial initiative was taken by the MIT Registry of Standard Biological Parts (http://partsregistry.org/Main_Page). A series of constitutive promoters (designated as BBa_J23100–BBa_J23119) have been designed and characterized. BBa_J23119 is the consensus promoter sequence and the strongest member of the family. BBa_J23112, BBa_J23113 and BBa_J23103 are weaker constitutive promoters that can be used in low level expressions of genes — especially toxic proteins. In this series, the two medium-strength constitutive promoters (BBa_J23105 and BBa_J23106), have been used for the construction of synthetic circuits