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Research review paper

Rapid prototyping of microbial cell factories via genome-scale engineering

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ABSTRACT

Advances in reading, writing and editing genetic materials have greatly expanded our ability to reprogram biological systems at the resolution of a single nucleotide and on the scale of a whole genome. Such capacity has greatly accelerated the cycles of design, build and test to engineer microbes for efficient synthesis of fuels, chemicals and drugs. In this review, we summarize the emerging technologies that have been applied, or are potentially useful for genome-scale engineering in microbial systems. We will focus on the development of high-throughput methodologies, which may accelerate the prototyping of microbial cell factories.

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1. Introduction

Microbial cell factories (MCFs), which convert biomass resources to value-added compounds such as fuels, chemicals, materials and pharmaceuticals, have been proposed as a sustainable and renewable alternative to the traditional petrochemical-based processes (Keasling, 2010; Lee et al., 2012; Rabinovitch-Deere et al., 2013). However, intensive reprogramming of cellular metabolism is required to achieve economically feasible fermentation processes with MCFs. Conventional strain engineering approaches rely on random mutagenesis, which is achieved through chemical mutagens/UV irradiation (Crook and Alper, 2012), prolonged cultivation under selective pressure (Portnoy et al., 2011), transposon insertions (Eckert et al., 2011; Hamer et al., 2001; Hutchison et al., 1999) and genome shuffling (Biot-Pelletier and Martin, 2014; Zhang et al., 2002). Effective in generating improved phenotypes using simple techniques, these methods are widely adopted in industry, especially for those host organisms with poorly defined genetics and limited engineering tools (Crook and Alper, 2012). However, traditional approaches are often labor-intensive, time-consuming, and difficult to analyze and transfer the genetic basis of a selected trait. Recently, the scale, efficiency and precision of genetic analysis and manipulation have been remarkably improved by several enabling technologies, including but not limited to microarray DNA synthesis, next-generation DNA sequencing (NGS), programmable DNA-binding proteins, and in vivo biosensors. Nowadays, billions of genome variants can be created in a directed and/or combinatorial manner, and the mutant strains with the optimal performance can be rapidly isolated. Collectively, these new technologies and their applications exemplify an emerging discipline called ‘genome engineering’ or ‘genome-scale engineering’ (Carr and Church, 2009; Esvelt and Wang, 2013; Jeong et al., 2013; Segal and Meckler, 2013).

The practice of genome-scale engineering can be broadly classified into three categories: genome editing, transcriptome engineering, and genome synthesis. Genome editing precisely or combinatorially modifies the target genome at multiple loci. Modifications are located either in the open-reading frames (ORFs) or in the *cis-acting* regulatory elements such as promoters and ribosome-binding sites (RBSs). Transcriptome engineering essentially targets *trans-acting* regulatory elements, such as transcription factors (TFs) or non-coding RNAs (ncRNAs), by mutating endogenous regulators or introducing artificial ones. Genome synthesis involves hierarchical assembly of short chemically synthesized DNA fragments into viral/microbial genomes and yeast chromosomes. Although current synthetic genomes are constructed mainly based on their wild type templates, the ultimate goal is to write genome sequences de novo.

In this review, we first introduce the recent development in genome editing (Genome editing section), transcriptome engineering (Transcriptome engineering section), and genome synthesis (Genome synthesis section). We then highlight how these techniques can facilitate high-throughput genotyping and phenotyping (High-throughput genotype–phenotype mapping section), which greatly accelerates our understanding and engineering of microbial genomes. In addition, we will discuss several examples on the application of genome-scale engineering to improve MCF performance and provide perspectives on how computational approaches and laboratory automation can be further integrated.

2. Genome editing

Unlike random mutagenesis, targeted genome editing results in elaborative and massive genome modifications with a traceable manner. Homologous recombination (HR) is the core mechanism of most targeted genome editing techniques, and various enzymes have therefore been investigated to either mediate or promote HR in microorganisms.

2.1. Recombinases

Recombinases catalyze exchange of short homologous regions (30–40 bp) of DNA. Site-specific recombinases are grouped into two families, the tyrosine recombinase family and the serine recombinase family (Turan et al., 2013). An early characterized member of the tyrosine recombinase family was λ integrase, which enables incorporation of phage DNA into the bacterial chromosomes. The λ integrase mediates irreversible recombination between the *attP* and *attB* sites in the phage and host chromosomes respectively, generating recombinant *attL* and *attR* sites (Mizuuchi and Mizuuchi, 1980). Later, Cre (from phage P1) and flippase (FLP, from the 2μ plasmid of yeast *Saccharomyces cerevisiae*), recognizing the *loxP* site and the flippase recognition target (FRT) site, respectively, were widely used for efficient recombination in a variety of species (Dymecki, 1996; Nagy, 2000; Sternberg et al., 1981; Turan et al., 2011). With identical recognition sites, Cre and FLP can reversibly invert, integrate or excise DNA sequences between recognition regions. Alternatively, such processes can be made irreversible using a partially mutated recognition site to yield a poorly recognized region after recombination (Albert et al., 1995; Schlake and Bode, 1994). For the serine recombinase family, ϕC31 integrase (from *Streptomyces* phage ϕC31) was the most well-studied example. Behaving like the λ integrase, ϕC31 was proven to have great potential in eukaryotic genome engineering (Karow and Calos, 2011).

In addition to inversion, integration and excision facilitated by the above-mentioned recombinases, recombinase-mediated cassette exchange (RMCE) is another useful approach in genome engineering. By flanking the target genomic locus with two different spacer mutant (“heterospecific”) sites recognized by the same recombinase or orthogonal sites of different recombinases, the endogenous region will be replaced by a donor cassette with compatible recognition sites (Turan et al., 2013). Many Cre and FLP variants were engineered to recognize different sites with little cross reactivity with the wild type system, allowing efficient directional cassette exchange (Fig. 1A) (Buchholz and Stewart, 2001; Schlake and Bode, 1994; Turan et al., 2010). By exploiting the specific *attP* × *attB* recombination event, ϕC31 was also applied to cassette exchange without the requirement of heterospecific *att*-sites. However, ϕC31 mediated-cassette change was in a unidirectional manner (Turan and Bode, 2011).

Notably, pre-existing recognition sites are required for all events mediated by recombinases. Therefore, introduction of recognition sites into the target locus is unavoidable, which limits the application of recombinase-based methods for genome editing. Although much effort has been invested in the directed evolution of recombinases with new target recognition sequences, the engineered enzymes were inefficient in most cases (Gordley et al., 2009).

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