Current Synthetic and Systems Biology

Singh and Braddick, Curr Synthetic Sys Biol 2015, 3:1
DOI:10.4172/2332-0737.1000e113

Editorial Open Access

Exploring the Potential of Small Regulatory RNA towards Microbial Engineering

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Rec Date: February 22, 2015, **Acc Date:** February 23, 2015, **Pub Date:** February 26, 2015

Abstract

Metabolic engineering has the potential to produce chemicals, fuels, drugs and more at industrial levels in a cost effective manner by manipulation of enzymatic, transport and regulatory function within cells. Small regulatory RNAs (sRNAs) play a key role in up and down regulation of genes associated with biosynthetic pathway for increasing production level. Therefore, sRNA is a rapid, sensitive and versatile tool for microbial engineering.

Keywords: Metabolic engineering; sRNA; Gene regulation; Device; Circuits

Editorial

Whilst microbes are an attractive chemical factory they are however often hampered by the inability to efficiently produce expected quantities of desired products. Therefore, the capability of natural microbes can be improved by the modification of the genome or changed by integration of foreign genes, which through expression of proteins in organism would lead to the production of desired compounds [1,2]. In many desired hosts either single gene or few genes are absent in the biosynthetic pathways of interest, and the bacteria cannot produce desired products. In this regard, metabolic engineering is the practice of designing of new biosynthetic pathways, entire synthetic genome [3], or improving the cellular activities of hosts by manipulation of enzymatic, transport and regulatory functions within cells to increase the cellular production of a desired product. Metabolic engineering has the potential to produce industrial levels of chemicals, fuels and drugs in a cost effective manner [2,4].

Escherichia coli is the most widely studied prokaryotic model organism and used in metabolic engineering and synthetic biology. It is a good choice due to its ease of culture, short life cycle, *well-known genetics and accessible tools.* In recent years, a number of synthetic parts that include promoters [5,6], regulatory proteins and RNAs [7-9], devices and circuits such as riboregulators [7], riboswitches [10,11], biologic gates [12,13], and oscillators [14-16] have been designed and characterized in a wide range of hosts. These synthetic networks have been implemented for rewiring [17-20], the coupling [21] of intracellular networks, or manipulating the cellular functions at certain scales that can be further useful for tight, tunable or periodic biological production.

In recent years, small regulatory RNAs (sRNAs) have become of greater scientific interest and they play a major role in gene regulation. The sRNAs can positively regulate translation of target mRNAs by binding to an upstream part of mRNA 5' untranslated regions and prevents formation of a translation-inhibitory hairpin structure. It opens the cis-repressed UTR and makes free RBS where ribosome binds and starts translation process (Figure 1a). Negative regulation by sRNAs typically involves base pairing interactions that occlude the ribosome binding sites (RBS) of mRNAs. This prevents translation either by mRNA repression or mRNA degradation (Figure 1b) [7,22,23]. Riboswitches are one of the most important sRNA forms that regulate gene expression in a ligand-dependent fashion. It works as a cis regulatory element which composes of an aptamer domain (recognizing the ligand) and an expression platform that couples ligand binding to a change in gene expression [24]. Riboregulators are also a form of sRNA that plays a pivotal role in up or down-regulating gene function. It controls the expression of target gene in trans at the post-transcriptional level [7,23,25,26].

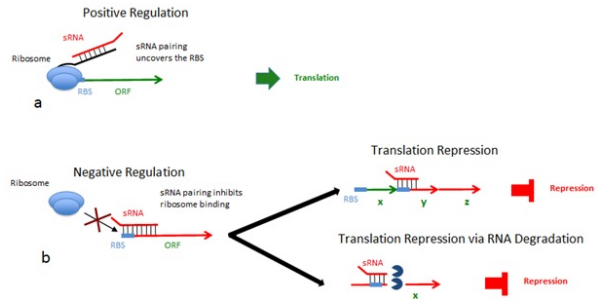

Figure 1: Schematic representation for the mode of action of sRNA. (a) Small RNAs can positively regulate translation of target mRNAs by binding to an upstream part of the mRNA 5' untranslated region thus preventing formation of a translation-inhibitory hairpin structure. (b) Negative regulation by sRNAs typically involves base pairing interactions that occlude the ribosome binding sites of mRNAs. This prevents translation either by mRNA repression or mRNA degradation.

This sRNA plays a number of regulatory functions in the cell including synthesizing proteins, splicing and editing RNA, modifying rRNA and catalyzing biochemical reactions. It belongs to a subset of non-coding RNAs that have emerged as important regulators in both prokaryotes and eukaryotes [7]. A number of studies have been focused on the identification, design, and characterization of sRNA for

better understanding of basic mechanism, gene regulation, enhanced tolerance and adaptation. The RNA chaperone Hfq helps sRNA efficiently binds to target mRNA genes in trans by base-pair complementation [23,27,28]. In studies of Hfq, the sRNAs were produced through plasmid-based expression to regulate the chromosomal gene expression, without direct modification of the chromosome sequence. This yields transient knock-down of gene regulation [23,26]. Recent reports on sRNA indicated that they may act as environmental sensors of vitamin cofactors and temperature, enabling them to transduce signals to regulate gene expression [24,29]. Regulatory RNAs operate by sensing environmental signals or other RNA molecules to either repress or activate translation [30].

Lease and Belfort [31] demonstrated that 87-nucleotide DsrA is a regulatory RNA *of E. coli* that acts in trans by RNA–RNA interactions with two different mRNAs, hns and rpoS. DsrA shows opposite effects on these transcriptional regulators and H-NS levels decrease, whereas RpoS (ss) levels increase. DsrA enhances hns mRNA turnover yet stabilizes rpoS mRNA, either directly or via effects on translation. In another study, Repoila et al. [32] reported the regulation of RpoS (σ38) translation as a function of the sRNA-mediated response to environmental conditions; rpoS is a gene known to be regulated post-transcriptionally by at least three sRNAs. DsrA and RprA stimulate RpoS translation in response to low temperature and cell surface stress, whereas OxyS represses RpoS translation in response to oxidative shock.

The sRNA Spot42 controls the synthesis of galactokinase (GalK) in response to the availability of glucose. SgrS sRNA represses the synthesis of glucose transporter EIIGlc and prevents the uptake of glucose when G6P accumulates towards toxic levels [33]. In recent years, phenol has become an industrially versatile chemical and is currently produced from fossil resources. A current total 18 *E. coli* strains have been engineered for the production of phenol using sRNAs. The sRNA used for knocking-down of the two regulators and for overexpression of the genes associated with the tyrosine biosynthetic pathway acts together with tyrosine phenol-lyase for the production of phenol from glucose [34]. The use of sRNAs could be useful for completely or transiently control the host genes and to enhance tolerance and/or high production levels. An urgent need is arising to identify and implement more sRNA that can be used in microbial engineering for high-level biological production. Na et al. [26] designed a library of small RNAs and employed them for increasing the production of tyrosine and the nylon precursor cadaverine. Considering these published applications, sRNA is considered as a rapid, sensitive and versatile tool for microbial engineering that can be useful for sufficient and cost effective biological production in the future to meet market demands at competitive prices.

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