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A real-time control system of gene expression using ligand-bound nucleic acid aptamer for metabolic engineering

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ABSTRACT

Artificial control of bio-functions through regulating gene expression is one of the most important and attractive technologies to build novel living systems that are useful in the areas of chemical synthesis, nanotechnology, pharmacology, cell biology. Here, we present a novel real-time control system of gene regulation that includes an enhancement element by introducing duplex DNA aptamers upstream promoter and a repression element by introducing a RNA aptamer upstream ribosome binding site. With the presence of ligands corresponding to the DNA aptamers, the expression of the target gene can be potentially enhanced at the transcriptional level by strengthening the recognition capability of RNAP to the recognition region and speeding up the separation efficiency of the unwinding region due to the induced DNA bubble around the thrombin-bound aptamers; while with the presence of RNA aptamer ligand, the gene expression can be repressed at the translational level by weakening the recognition capability of ribosome to RBS due to the shielding of RBS by the formed aptamer-ligand complex upstream RBS. The effectiveness and potential utility of the developed gene regulation system were demonstrated by regulating the expression of *ecaA* gene in the cell-free systems. The realistic metabolic engineering application of the system has also tested by regulating the expression of *mgtC* gene and thrombin cDNA in *Escherichia coli* JD1021 for controlling metabolic flux and improving thrombin production, verifying that the real-time control system of gene regulation is able to realize the dynamic regulation of gene expression with potential applications in bacterial physiology studies and metabolic engineering.

1. Introduction

Recent technological breakthroughs in metabolic engineering have made it easier to overproduce biochemical products from renewable resources (Tong et al., 2001; Benner and Sismour, 2005; Zhang et al., 2009). The main target in new engineering metabolic pathways is to maximize the metabolic flux for achieving the optimum yield of the desired product (Krishnamurthy et al., 2015). Novel genetic regulatory systems contained in synthetic gene circuits have emerged as powerful tools to control the metabolic flux *via* fabricating large synthetic pathway (Iyer and Doktyca, 2014; Cox et al., 2007), and optimizing pathway expression through the transcription-level (*e.g.* constitutive, inducible promoters with high dynamic ranges) (Cox et al., 2007; J. Wang et al., 2015a; Z.P. Wang et al., 2015b) or translation-level engineering (*e.g.* RNAi regulation system; RNA-based gene regulation system; libraries of RBS) (Krishnamurthy et al., 2015; Sakai et al., 2014; Egbert and Klavins, 2012). These approaches, nevertheless, may not necessarily yield the best results as most of the gene regulation systems can only regulate gene expression at a fixed level throughout the metabolism, during which, the most suitable expression levels of key genes are different in different processes. Therefore, unnecessary or insufficient expression of key genes in some specific processes could cause metabolic burdens or poor metabolism that interferes with the need of cells for basic maintenance and propagation, significantly reducing the yield of the target products (Wang et al., 2016; Klein et al., 2014). In addition, a large portion of gene regulation systems that were predicted theoretically to perform well execute poorly in practice; and most of the systems cannot withstand the change of culture conditions when being

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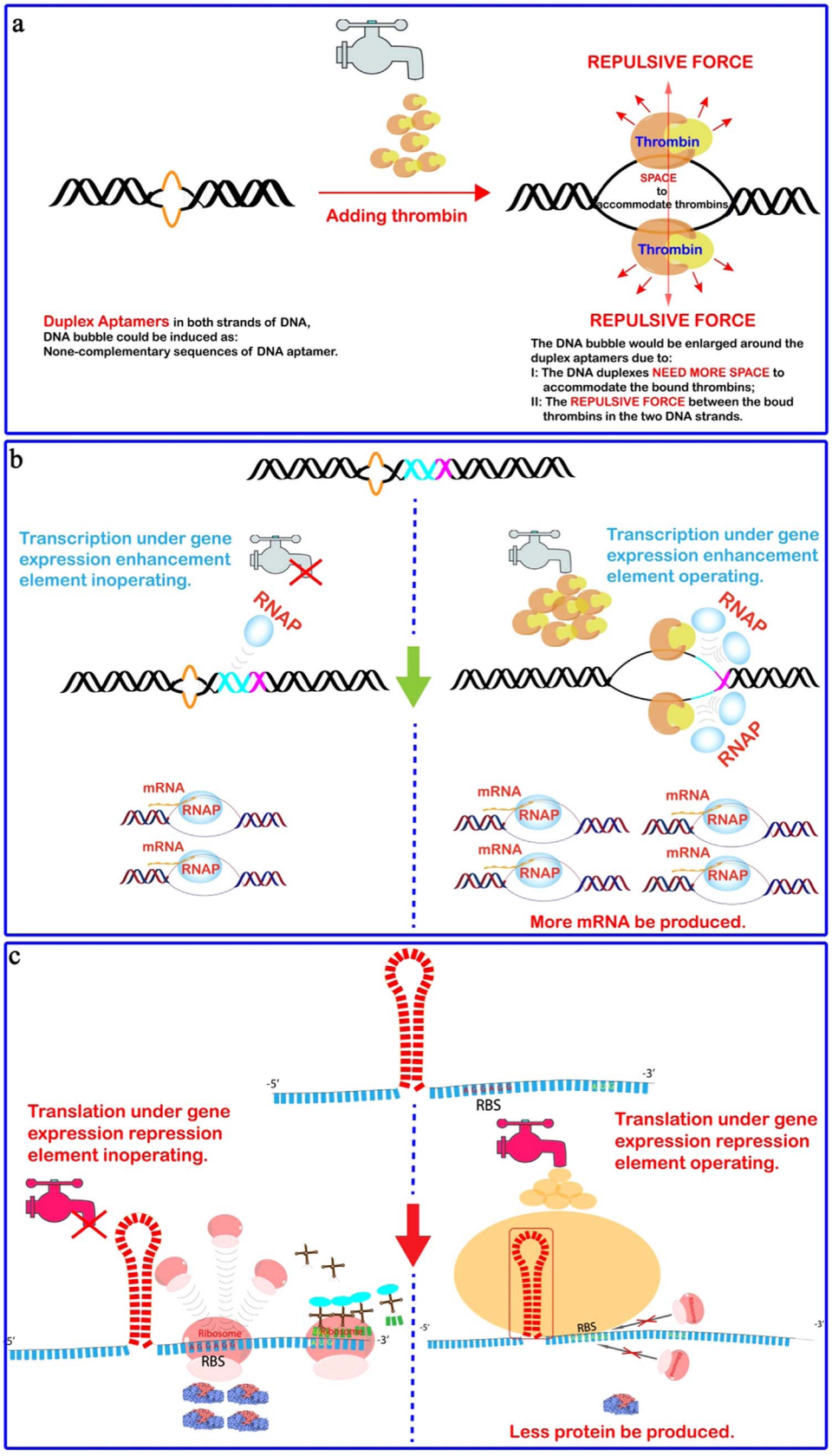

Fig. 1. A DNA bubble can be induced by introducing duplex aptamers upstream promoter with the existence of thrombin, enhancing gene expression at the transcriptional level. (a) The DNA bubble can only be induced by introducing duplex aptamers upstream promoter and with the existence of specific ligand (thrombin in this study). (b) The recognition region of the promoter contained in the DNA bubble can be more easily recognized and bound by RNAPs, and the separation of the unwinding region of the promoter contained in the DNA bubble can also be accelerated, leading to the enhanced expression of the target gene at the transcriptional level. (c) A VEGF RNA aptamer was introduced upstream RBS of the target gene. With the presence of VEGF, VEGF would bind to the RNA aptamer, resulting in the shielding of RBS by the formed aptamer-ligand complexes upstream RBS. The shielded RBS is harder to be recognized and bound by ribosome, leading to the repressed expression of the target gene at translational level.

transferred to other species and environment since the current molecular tools are strictly specific in hosts and conditions (ZP Wang et al., 2015; J Wang et al., 2015). Consequently, it is challenge to build a regulatory system with a protein sensor that can measure key intermediates in the synthesis cascade and cognate regulators that can control gene expression to improve production of the desired chemicals.

Nature has evolved various sensors for intracellular molecules that could be used to sense the biosynthetic intermediates. The ability to