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Transcription control engineering and applications in synthetic biology

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ABSTRACT

In synthetic biology, researchers assemble biological components in new ways to produce systems with practical applications. One of these practical applications is control of the flow of genetic information (from nucleic acid to protein), a.k.a. gene regulation. Regulation is critical for optimizing protein (and therefore activity) levels and the subsequent levels of metabolites and other cellular properties. The central dogma of molecular biology posits that information flow commences with transcription, and accordingly, regulatory tools targeting transcription have received the most attention in synthetic biology. In this mini-review, we highlight many past successes and summarize the lessons learned in developing tools for controlling transcription. In particular, we focus on engineering studies where promoters and transcription terminators (*cis*-factors) were directly engineered and/or isolated from DNA libraries. We also review several well-characterized transcription regulators (*trans*-factors), giving examples of how *cis*- and *trans*-acting factors have been combined to create digital and analogue switches for regulating transcription in response to various signals. Last, we provide examples of how engineered transcription control systems have been used in metabolic engineering and more complicated genetic circuits. While most of our mini-review focuses on the well-characterized bacterium *Escherichia coli*, we also provide several examples of the use of transcription control engineering in non-model organisms. Similar approaches have been applied outside the bacterial kingdom indicating that the lessons learned from bacterial studies may be generalized for other organisms.

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1. Introduction

A central tenet of synthetic biology is the ability to assemble a practical system from biological components. In most cases, the components are proteins synthesized from genetic information encoded in DNA. One of the challenges faced by synthetic biologists is designing the appropriate genetic sequence to control protein expression at the level that optimizes system function. The central dogma of molecular biology (Fig. 1) describes the flow of genetic information from nucleic acid polymers (DNA and RNA) to amino acid polymers (proteins) [1]. Transcription is the process during which information encoded in DNA is converted to a transient carrier of information, mRNA. Translation is the process in which the mRNA code is converted to an amino acid polymer by ribosomes, themselves RNA catalysts (ribozymes) [2–5]. The flow of genetic information terminates with the production of a protein, but often post-translational modifications and processes (see below) are needed to produce the final functional component. To optimize the abundance and other properties of the protein and the performance of the ultimate application, the rate and timing of each of these processes can be controlled by both *cis*-acting (within or adjacent to the protein coding sequence) and *trans*-acting (separate from the protein coding sequence) components.

Transcriptional control is a prevalent mode of regulating protein production because it is the first step in the process. Accordingly, it has received the most attention in terms of tool development and engineering applications. Transcriptional regulation allows a cell to allocate its valuable resources towards production of proteins when

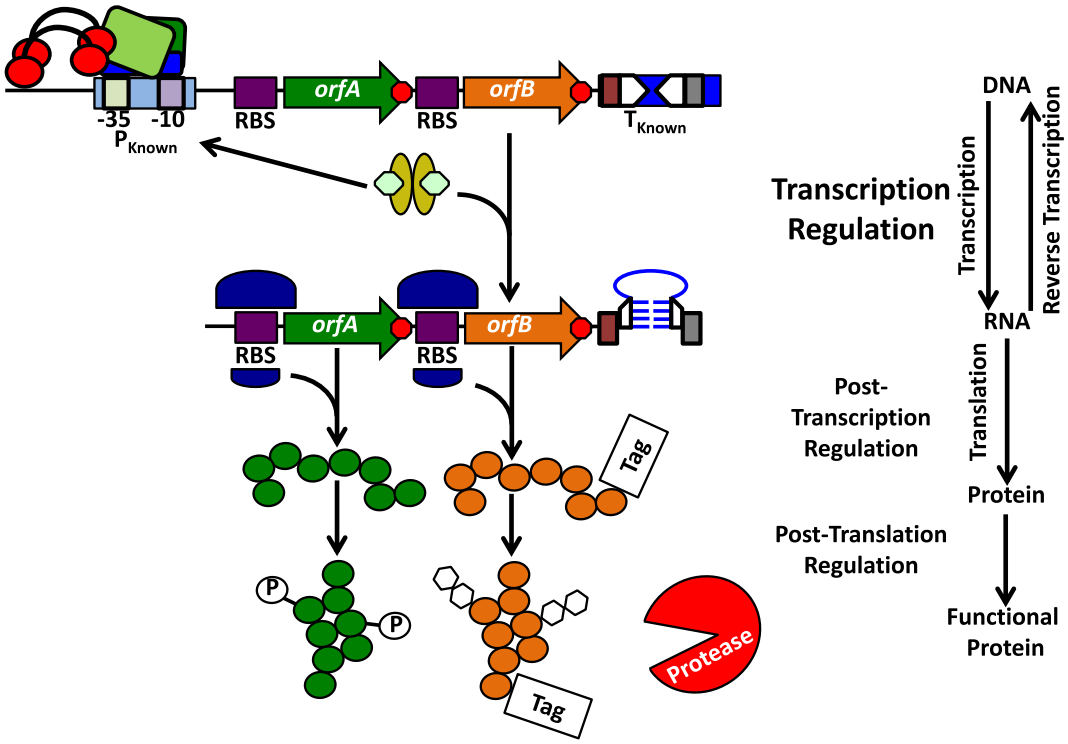

Fig. 1. Overview of the central dogma of molecular biology. An operon encoding *orfA* and *orfB* is under the control of the indicated promoter (P_{Known}), which is engaged by the RNA polymerase holoenzyme (green and blue boxes, red circles, and black arcs). Sequences that will be transcribed into the ribosome binding sites (RBSs) for *orfA* and *orfB* (purple boxes) and the transcription terminator (T_{Known}; two inverted repeats [inverted arrowheads] flanked by a poly A-tract [red box] and poly U-tract [grey box]) are indicated. Red octagons represent translation stop codons. At the level of ***transcription regulation***, alterations to the promoter and terminator sequences, the presence of transcription activators and repressors (depicted as golden ovals with green hexagon ligands bound), and nucleoid/supercoiling states may all influence *orfA* and *orfB* transcription (not shown). Through the process of transcription itself, the associated *orfA* and *orfB* mRNA is produced, which may in turn be bound by ribosomes (depicted as two variable size purple half circles) at the RBS. To terminate transcription, the depicted terminator sequence forms a stem-loop (blue), mediated by base pairing/stacking with the complementary sequences of the former inverted repeats (half arrowheads), and mRNA synthesis ceases at this site (poly A- and ploy U-tracts are indicated as above). Modifying RBS strength, RNA riboswitches, aptazymes (aptamer ribozymes), and RNA stability may each contribute to ***post-transcription regulation*** (not shown). Folding of a translated protein (green and orange circles) with the assistance of folding chaperones (not shown), enzymes that promote post-translation modifications such as phosphorylation (P in a white circle) or glycosylation (white hexagons), the presence of proteolytic enzymes (indicated in the figure and potentially targeted to a protein through a specific motif [Tag]) represent methods of ***post-translation regulation***.

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