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Understanding and exploiting feedback in synthetic biology

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HIGHLIGHTS

- There are important distinctions between feedback in process control and in biology.
- Negative, positive, and combined feedback exhibit unique properties.
- Feedback can be readily applied to advance the construction of biological systems.

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ABSTRACT

Synthetic biology employs traditional engineering concepts in the construction of cells and organisms. One of the most fundamental concepts is feedback, where the activity of a system is influenced by its output. Feedback can imbue the system with a range of desirable properties such as reducing the rise time or exhibiting an ultrasensitive response. Feedback is also commonly found in nature, further supporting the incorporation of feedback into synthetic biological systems. In this review, we discuss the common attributes of negative and positive feedback loops in gene regulatory networks, whether alone or in combination, and describe recent applications of feedback in metabolic engineering, population control, and the development of advanced biosensors. The examples principally come from synthetic systems in the bacterium *Escherichia coli* and in the budding yeast *Saccharomyces cerevisiae*, the two major workhorses of synthetic biology. Through this review, we argue that biological feedback represents a powerful yet underutilized tool that can advance the construction of biological systems.

1. Introduction

The field of synthetic biology aims to construct biological systems in order to better understand nature and to address pressing challenges facing society. However, the inherent complexity of biology impedes the construction of predictable and robust systems. Fortunately, concepts in traditional engineering disciplines offer approaches to reduce biological complexity and subsequently advance biological design.

One engineering discipline that has strongly influenced synthetic biology is process control. This discipline seeks to automate industrial processes in order to maintain system specifications with limited oversight. One prevalent tool in process control is feedback. Feedback serves multiple purposes, including driving the output of a system toward a desired setpoint, countering disturbances in system inputs, filtering measurement noise, and decoupling relationships between multiple inputs and multiple outputs. The physical link between system inputs and outputs is called a feedback loop. Process control principally focuses on negative feedback loops that drive the system output toward the setpoint. Positive feedback loops, which drive the system output away from the setpoint, are adopted less frequently (Horowitz and Hill, 1989).

Both negative and positive feedback loops can be found throughout the architecture of gene regulatory networks. Extensive studies, particularly in the field of systems biology, have revealed that these biological feedback loops shape the dynamics, variability, and steady-state response of the system. These influences in turn have been implicated in the adaptability and robustness of biological systems. The mechanisms of feedback vary widely and can be combined, resulting in overlaid negative and positive feedback loops with unique properties.

Despite the many benefits of feedback in biology and its prevalence in other engineering disciplines, synthetic biology has been slow to implement feedback in the design of biological systems. Arguably, the field is still grappling with how to construct large-scale systems that behave predictably and has not yet advanced to the point of including additional layers of control. As a result, recent work has centered on the construction of logic gates with either higher-order processing or the ability

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to interpret multiple signals (Rinaudo et al., 2007; Friedland et al., 2009; Tamsir et al., 2011; Moon et al., 2012). Feedback has been integrated into a handful of synthetic biological systems, but often only in the cases in which feedback was essential for the desired system behavior (e.g. genetic oscillators (Elowitz and Leibler, 2000; Atkinson et al., 2003; Fung et al., 2005; Stricker et al., 2008)). However, feedback offers a wealth of attributes that can generate more desirable behaviors and improve the robustness of biological systems.

In this review, we discuss the known properties of negative and positive feedback loops – both in isolation and in combination – in biological systems. Examples principally are drawn from bacteria and yeast, the current workhorses of synthetic biology. Many of the insights into feedback were drawn from synthetic systems, demonstrating how synthetic biology informs our understanding of nature. We then discuss recent applications of feedback loops in synthetic biology. Finally, we conclude by describing how feedback can be further implemented to advance current applications in the field.

2. Modes of feedback

Feedback can occur at multiple steps of gene expression or through the interactions between organisms. Fig. 1 illustrates representative mechanisms through which feedback can be introduced. We touch on many of these mechanisms in discussing the properties and application of feedback in biology. Note that even simpler examples of feedback often engage multiple mechanisms at one time.

2.1. Transcriptional regulation

In the first step of gene expression, messenger RNA is transcribed from the DNA of a gene. This step begins with transcription factors recruiting RNA polymerase to the gene's promoter and ends with termination and release of the polymerase. Both steps offer opportunities for both negative and positive feedback, by controlling the accessibility or methylation of the DNA (Lim and Van Oudenaarden, 2007; Octavio et al., 2009), the availability of RNA polymerase or transcription factors, or transcriptional termination (Winkler et al., 2002; Lucks et al., 2011). The simplest form of transcriptional feedback, called auto-regulation, involves a transcription factor regulating its own transcription. Auto-regulation is one of the most common transcriptional architectures found in bacteria (Shen-Orr et al., 2002) and has undergone the most extensive characterization out of all modes of feedback.

2.2. Post-transcriptional regulation

Following transcription, the messenger RNA is translated into protein. In microorganisms, this step can be regulated by modulating RNA stability and translation. In most cases, the responsible mechanisms involve the interaction of a trans-acting factor. This factor could be a protein, such as a ribonuclease or the RNA binding protein CsrA; an RNA, such as Hfq-binding small RNAs or synthetic riboregulators; or a small molecule, such as cofactors recognized by translational riboswitches (Waters and Storz, 2009). Many of the RNA-based mechanisms are still undergoing characterization and have not been quantitatively studied in the context of feedback.

2.3. Post-translational regulation

Once a protein is formed, feedback can be introduced by modulating the protein's stability, localization, or activity. Stability can be modulated by attaching a degradation tag or altering protease activity, influencing protein levels. Next, localization can be modulated by targeting the protein to the cell membrane or to an organelle, affecting whether the protein can access its target. Finally, protein activity can be modulated through chemical modifications or reversible binding of small molecules, RNAs, or proteins ((Liu et al., 1997; Wassarman and Storz, 2000; Buchler and Cross, 2009; Hunsicker et al., 2009), altering the ability of the protein to carry out its normal functions. Modulating protein activity is the most common post-translational mechanism of

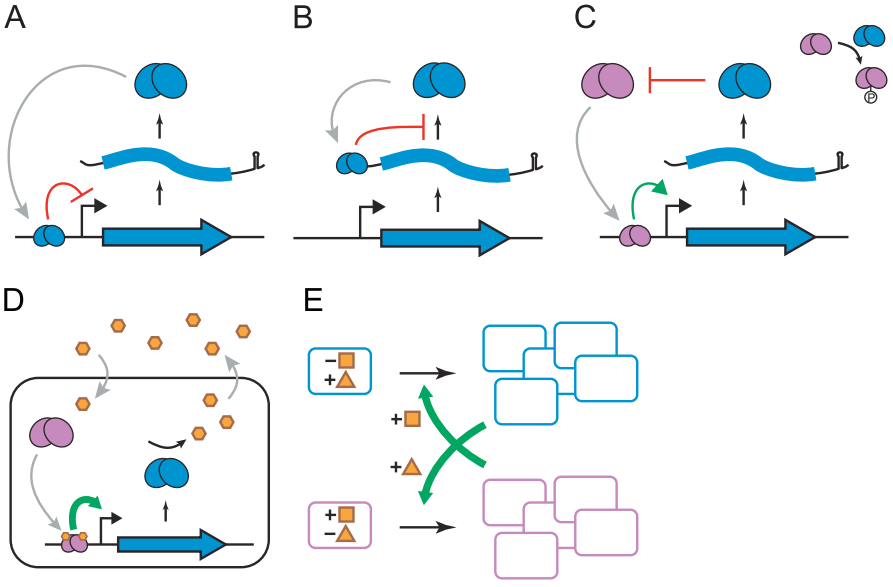

Fig. 1. Modes of feedback in microorganisms. Feedback can be introduced at the different steps of gene expression (A)–(C) and through the interactions between cells (D) and (E) Red block lines designate negative regulation while green arrows designate positive regulation. (A) Transcriptional regulation, as shown for auto-repression. (B) Post-transcriptional regulation, as shown for an RNA-binding protein inhibiting its own translation. (C) Post-translational regulation, as shown for phosphorylation of a transcription activator that inhibits DNA binding. (D) and (E) Cell–cell interactions, as shown for the synthesis and secretion of a diffusible molecule that activates the expression of the synthesis enzyme (D), and for a mutualistic interaction where one strain produces an essential metabolite not produced in the other strain (E). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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