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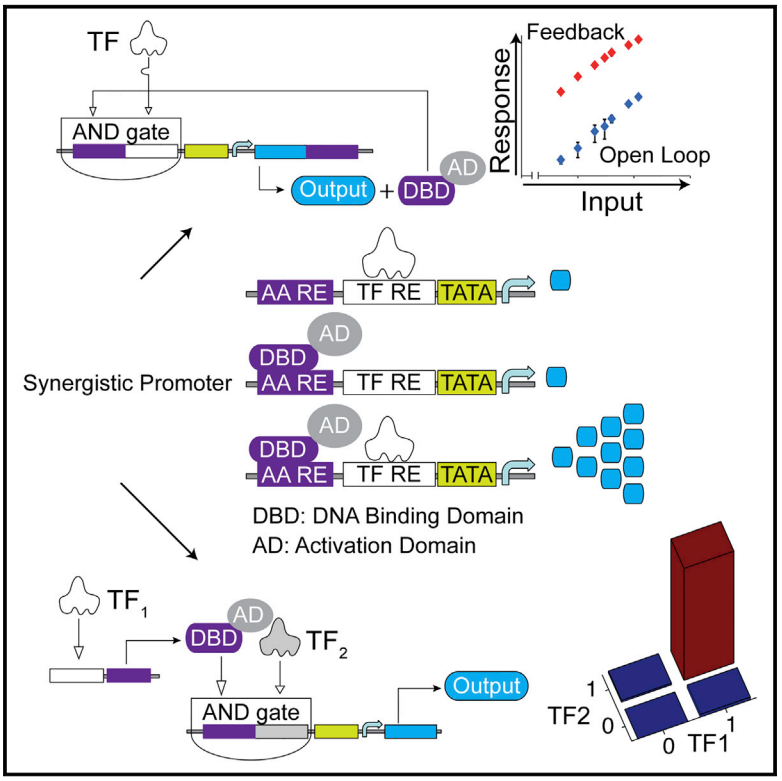
Synthetic Biology Platform for Sensing and Integrating Endogenous Transcriptional Inputs in Mammalian Cells

Graphical Abstract



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In Brief

Coupling endogenous transcription factor activities to synthetic gene circuits has been a longstanding challenge. Angelici et al. describe highly synergistic composite promoters that enable robust and selective amplification of mammalian transcriptional inputs using positive feedback as well as their arbitrary pairing in promoter-level AND gates. The resulting sensors can efficiently transduce their inputs' signal to downstream synthetic circuits via a variety of mechanisms, including RNAi, transactivation, and recombination.

Highlights

- A positive feedback loop with high synergy between trigger input and amplifier
- Rule-based design of robust amplified sensors of mammalian transcription factors
- AND gates between pairs of unrelated transcription factors
- Efficient transduction of transcriptional inputs into diverse downstream actuation

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Synthetic Biology Platform for Sensing and Integrating Endogenous Transcriptional Inputs in Mammalian Cells

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SUMMARY

One of the goals of synthetic biology is to develop programmable artificial gene networks that can transduce multiple endogenous molecular cues to precisely control cell behavior. Realizing this vision requires interfacing natural molecular inputs with synthetic components that generate functional molecular outputs. Interfacing synthetic circuits with endogenous mammalian transcription factors has been particularly difficult. Here, we describe a systematic approach that enables integration and transduction of multiple mammalian transcription factor inputs by a synthetic network. The approach is facilitated by a proportional amplifier sensor based on synergistic positive autoregulation. The circuits efficiently transduce endogenous transcription factor levels into RNAi, transcriptional transactivation, and site-specific recombination. They also enable AND logic between pairs of arbitrary transcription factors. The results establish a framework for developing synthetic gene networks that interface with cellular processes through transcriptional regulators.

INTRODUCTION

Development of synthetic gene networks, or circuits, has tended to employ two complementary approaches. One approach focuses on the unique function of a gene circuit, e.g., biomanufacturing (Steen et al., 2010), complex dynamics (Elowitz and Leibler, 2000; Gardner et al., 2000; Stricker et al., 2008; Tigges et al., 2009), or information processing (Ausländer et al., 2012; Benenson, 2012; Deans et al., 2007; Friedland et al., 2009; Green et al., 2014; Park et al., 2003; Rinaudo et al., 2007; Tamsir et al., 2011). The cells in which these networks operate are viewed as relatively passive containers, or chassis. A complementary effort aims to alter or modify cellular processes by focusing on gene circuits that interface with the host cell, sensing endogenous inputs from the cell or environment and responding with specific biologically active outputs (Ausländer et al., 2014; Culler et al., 2010; Kobayashi et al., 2004; Nissim and Bar-Ziv, 2010; Slomovic and Collins, 2015; Xie et al., 2011). Such circuits are conceptually similar to regulatory or signaling pathways, with inputs typically conveying information about an internal or environmental cell state and thus driving a desired response.

Although known mechanisms are typically used to establish interactions between endogenous inputs and synthetic components, extensive engineering effort is often necessary to match the two. One example is a family of proportional microRNA (miRNA) sensors (Lapique and Benenson, 2014) that employ RNAi (Fire et al., 1998; McManus and Sharp, 2002). Mammalian transcription factors (TFs) comprise another family of well-studied (Janknecht et al., 1993; Kadonaga et al., 1987), information-rich cellular inputs (Hobert, 2008). Although researchers constructed used complex transcriptional regulatory building blocks and networks (Amit et al., 2011; Farzadfard et al., 2013; Khalil et al., 2012; Leisner et al., 2010; Li et al., 2015; Lienert et al., 2013; Maeder et al., 2013; Perez-Pinera et al., 2013), they have tended to employ non-native transcriptional inputs.

Here, we present a framework for systematic rational design of selective and robust sensing, integration, and transduction of endogenous TF activity in mammalian cells. We begin by describing a cell-based assay for characterization of TF sensor elements and their comparative analysis. We use five transcriptional activators, each tested with a panel of response elements (REs). Due to modest induction levels, we augmented the sensors with positive transcriptional feedback using an artificial amplifier activator and observed, counterintuitively, high response levels and low leakage. We dissect the behavior of composite promoters within this feedback loop and uncover high synergy between the feedback amplifier activator and the endogenous input of interest. As a result, the sensors do not function as binary switches (Xiong and Ferrell, 2003) that generate either very low or saturated output, depending on whether the input is below or above a certain threshold (all-or-none response). Instead, they are amplifiers whose output grows in proportion to the input. They operate well within the physiological activity range of the input. The initial dataset and computational analysis allow formulation of design principles that we illustrate using three additional TFs. We next show that high-synergy promoters can be employed for tunable two-input AND logic between unrelated TFs, requiring simultaneous activation by both factors to trigger a response. Furthermore, we demonstrate