Emergent bistability by a growth-modulating positive feedback circuit

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Abstract — A synthetic gene circuit is often engineered by **considering the host cell as an invariable "chassis". Circuit** activation, however, may modulate host physiology, which in turn can drastically impact circuit behavior. We illustrate this point by a simple circuit consisting of mutant T7 RNA polymerase (T7 RNAP*) that activates its own expression in bacterium *Escherichia coli*. Although activation by the T7 RNAP* is noncooperative, the circuit caused bistable gene expression. This counterintuitive observation can be explained by growth retardation caused by circuit activation, which resulted in nonlinear dilution of T7 RNAP* in individual bacteria. Predictions made by a model accounting for such effects were verified by further experimental measurements.

Keywords — bistability, host physiology, positive feedback, synthetic biology.

I. Introduction

A central challenge in synthetic biology is to construct reliable and useful biological systems in a predictable manner [1]. A typical design process entails multiple rounds of gene circuit modeling, construction, and optimization. This process is often carried out by considering the host cell as an invariable "chassis", or assuming a well-defined interface with the circuit. This circuit-centric view of gene circuit engineering has been implied in efforts to standardize synthetic biological parts [2].

As has been noted, however, a circuit is functional only in the context of its host and its activation may invoke unintended interactions with the host [3]. These interactions may be local: there may be cross-talk between circuit components and endogenous host proteins. They may be global: expression of circuit components may be detrimental or beneficial to the host cell, leading to modulation of cell physiology. In general, unintended interactions have been neglected in engineering and characterizing synthetic gene circuits. This practice is advantageous in that it can drastically simplify the design process. To date, it appears to be well-justified in published examples, where the dominant observed dynamics can be explained by the intended circuit design.

However, interactions between a circuit and the host physiology may produce additional feedback regulation that significantly changes designed dynamics, as suggested in studies of signaling networks [4, 5]. In efforts to engineer gene circuits to date, however, little attention has been paid to the impact of such unintended circuit-host interactions. Specifically, it remains elusive as to how such interactions modulate dynamic of nonlinear gene circuits. A better understanding of this question has profound implications for exploring design strategies of biological networks, for standardizing biological parts and systems, and for engineering cells as computing units [6].

II. Conclusion

Our results reveal a hereto-unknown mechanism by which bistability arises from the interplay between a noncooperative positive feedback circuit and circuit-induced growth retardation, which has implications for analyzing both synthetic and natural circuits. This mechanism is fundamentally distinct from the previously identified mechanisms, which have focused on generation of nonlinear behavior by the circuit *per se* [7]. Moreover, it underscores the critical need to consider the modulation of host physiology when engineering and characterizing synthetic gene circuits.

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