The development of RNA-based control systems to regulate signaling and dictate cell fate in a model MAPK pathway

Thesis by

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Abstract

Cells integrate extracellular information via native signaling pathways to spatially and temporally coordinate complex tasks such as development and the immune response. Cellular programming holds the potential of harnessing the sophisticated and complex biological processes of living cells for diverse applications. In the last decade, cellular reprogramming has emerged as a viable therapeutic strategy. In large, reprogramming strategies have relied on statically programmed levels of gene expression to alter cellular behaviors. To construct more sophisticated programs requires dynamic control of expression and strategies for the facile construction of complex control architectures. Additionally, the application of synthetic programs to the control of native regulatory pathways requires the development of tools for interfacing with these pathways, as well as the construction of stringent controllers. Further, control systems composed of modular and tunable elements will facilitate the expansion of synthetic circuitry to a wide array of natural networks with varying system properties.

Here we describe the development of RNA-based control systems to regulate signaling and dictate cell fate in a model mitogen-activated protein kinase (MAPK) pathway. We construct networks of RNA-based control systems that interface with the *Saccharomyces cerevisiae* mating pathway to dictate entry into one of three programmed alternative fates dependent on environmental stimuli. We present a readily translatable method for identifying control points within natural networks that enable the construction of a modular interface between synthetic circuitry and native networks. In building these networks, we demonstrate the rational tuning of circuit performance via the exchange of well-defined parts to compose networks capable of actuating changes in cellular behavior

in response to environmental cues. Further, we construct network architectures which facilitate reduced interference from simultaneously integrated opposing programs and identified sensitive parameters for engineering robust circuit performance. Finally, we present the development of a novel RNA-based control element for the regulation of both synthetic and endogenous transcripts. This work provides a model for engineering systems that regulate signaling and direct cell fate which may be applied to additional decision-making pathways to advance tissue engineering strategies, treat diseases, and study the behavior of natural regulatory networks.

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