Dynamically Reshaping Signaling Networks to Program Cell Fate via Genetic Controllers

Kate E. Galloway, Elisa Franco, Christina D. Smolke*

READ THE FULL ARTICLE ONLINE
http://dx.doi.org/10.1126/science.1235005

Cite this article as K. E. Galloway *et al.*, *Science* **341**, 1235005 (2013).
DOI: 10.1126/science.1235005

Introduction: Engineering of cell fate through synthetic gene circuits requires methods to precisely implement control around native decision-making pathways and offers the potential to direct developmental programs and redirect aberrantly activated cell processes. We set out to develop molecular network diverters, a class of genetic control systems, to activate or attenuate signaling through a mitogen-activated protein kinase (MAPK) pathway, the yeast mating pathway, to conditionally route cells to one of three distinct fates.

Methods: We used a combination of genetic elements—including pathway regulators, RNA-based transducers, and constitutive and pathway-responsive promoters—to build modular network diverters. We measured the impact of these genetic control systems on pathway activity by monitoring fluorescence from a transcriptional pathway reporter. Cell fate determination was measured through halo assays, in which mating-associated cell cycle arrest above a certain concentration of pheromone from wild-type cells results in a "halo" or cleared region around a disk saturated in pheromone. A phenomenological model of our system was built to elucidate design principles for dual diverters that integrate opposing functions while supporting independent routing to alternative fates.

Results: We identified titratable positive (Ste4) and negative (Msg5) regulators of pathway activity that result in divergent cell fate decisions when controlled from network diverters. A positive diverter, controlling Ste4 through a feedback architecture, routed cells to the mating fate, characterized by pathway activation in the absence of pheromone. A negative diverter, controlling Msg5 through a nonfeedback architecture, routed cells to the nonmating fate, characterized by pathway inhibition in the presence of pheromone. When integrated into a dual-diverter architecture, the opposing functions of these positive and negative diverters resulted in antagonism, which prevented independent routing to the alternative fates. However, a modified architecture that incorporated both constitutive and feedback regulation over the pathway regulators enabled conditional routing of cells to one of three fates (wild type, mating, or nonmating) in response to specified environmental signals.

Discussion: Our work identified design principles for networks that induce differentiation of cells in response to environmental signals and that enhance the robust performance of integrated mutually antagonistic genetic programs. For example, integrated negative regulators can buffer a system against noise amplification mediated through positive-feedback loops by providing a resistance to amplification. Negative feedback can play an important role by reducing population heterogeneity and mediating robust, long-term cell fate decisions. The dual-diverter configuration enables routing to alternative fates and minimizes impact on the opposing diverter by integrating differential regulatory strategies on functionally redundant genes. Molecular network diverters provide a foundation for robustly programming spatial and temporal control over cell fate.

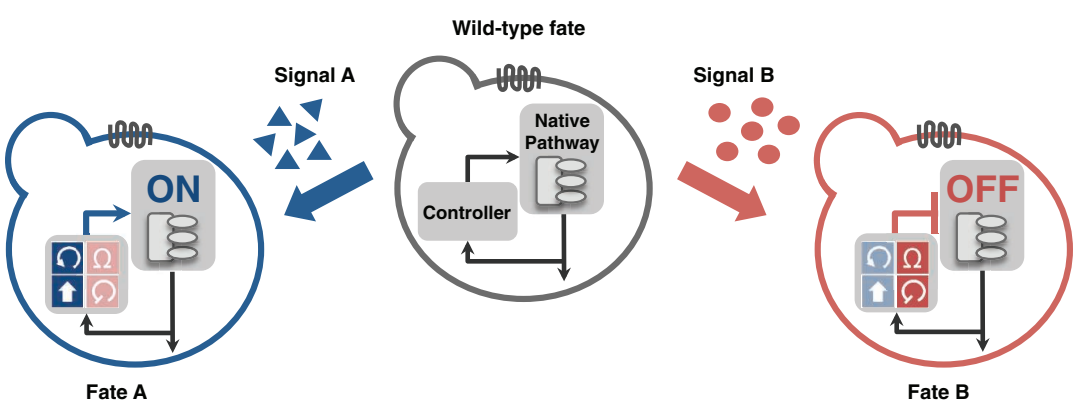
FIGURES IN THE FULL ARTICLE

SUPPLEMENTARY MATERIALS

Materials and Methods
Supplementary Text
Figs. S1 to S14
Tables S1 to S17
References

RELATED ITEMS IN *SCIENCE*

C. A. Sarkar, Concentrating (on) natural signaling proteins for synthetic control of cell fate. *Science* **341**, 1349–1350 (2013).
DOI: 10.1126/science.1243994



The molecular network diverter (controller) interfaces with a native signaling pathway to conditionally route cells to one of three fates in response to distinct environmental signals. Signal A (**left**) and signal B (**right**) trigger the positive and negative elements of the diverter via their cognate transducers to activate or inhibit, respectively, signaling through the yeast mating pathway.

The list of author affiliations is available in the full article online.
*Corresponding author. E-mail: christina.smolke@stanford.edu