

RNA-based control systems and their application to a model MAPK pathway

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Novel cellular behaviors can be generated by coupling engineered control systems to the cell's natural regulatory network [1]. However, the ability to run pre-programmed control algorithms depends on the facile creation of modular input and output interfaces between endogenous and exogenous systems. Currently, engineering gene circuits relies heavily on engineering protein-DNA interactions to control transcription. The rational design of these molecules and their interaction has been impeded by our limited understanding of how to engineer precise tertiary structures and function into proteins. Until it is possible to create *de novo* connections between gene expression elements and regulators, the application of cellular programming strategies will be limited by a small set of input and output interfaces. As a result of these limitations, widespread application of exogenous control to cellular engineering applications remains unfeasible. The modular and tunable nature of RNA makes it an ideal candidate to perform both sensing and actuation functions within an exogenous control system [2].

Recent studies have demonstrated the design and implementation of synthetic small molecule-regulated RNA switches by pairing aptamers with expression platforms [3-5]. Synthetic riboswitches take advantage of a wealth of expression platforms from antisense [4] to ribozymes [6]. Synthetic riboswitches are comprised of a sensor domain connected to an actuator domain through an engineered linking module that allows communication between the two elements. I am developing and characterizing *trans*-acting ribozymes to act as regulators of endogenous gene expression. Through the rational coupling of aptamers and *trans*-acting ribozyme domains, I plan to construct and characterize *trans*-ribozyme switches that behave as molecular control elements, regulating gene expression of target transcripts in response to exogenous and endogenous ligands.

RNA-based control systems will be used to probe the open-loop dynamics of the yeast pheromone-responsive MAPK pathway and develop closed-loop strategies. MAPK cascades are highly conserved signaling pathways that control numerous cellular processes such as differentiation, mitosis, and apoptosis [7]. The question of how specificity is conferred in networks with redundant parts is directly tied to the issue of control in MAPK cascades. Researches are already looking into the function of feedback loops and levels of transcription factors to elucidate the details of signal specificity [8]. Phenotypic outcome appears to be tied to MAPK levels. Control systems that dynamically regulate gene expression offer the unique opportunity to specify protein levels and thereby prescribe phenotype. The ability to control signaling through the yeast pheromone MAPK cascade will significantly improve our capacity to prescribe cellular behavior and dictate cellular fate.

1. Kobayashi, H., et al., *Programmable cells: interfacing natural and engineered gene networks*. Proc Natl Acad Sci U S A, 2004. **101**(22): p. 8414-9.
2. Isaacs, F.J., D.J. Dwyer, and J.J. Collins, *RNA synthetic biology*. Nat Biotechnol, 2006. **24**(5): p. 545-54.

3. Winkler, W.C. and R.R. Breaker, *Genetic control by metabolite-binding riboswitches*. *ChemBiochem*, 2003. **4**(10): p. 1024-32.
4. Bayer, T.S. and C.D. Smolke, *Programmable ligand-controlled riboregulators of eukaryotic gene expression*. *Nat Biotechnol*, 2005. **23**(3): p. 337-43.
5. Buskirk, A.R., A. Landrigan, and D.R. Liu, *Engineering a ligand-dependent RNA transcriptional activator*. *Chem Biol*, 2004. **11**(8): p. 1157-63.
6. Win, M.N. and C.D. Smolke, *A modular and extensible RNA-based gene-regulatory platform for engineering cellular function*. *Proc Natl Acad Sci U S A*, 2007.
7. Seger, R. and E.G. Krebs, *The MAPK signaling cascade*. *Faseb J*, 1995. **9**(9): p. 726-35.
8. Esch, R.K., Y. Wang, and B. Errede, *Pheromone-induced degradation of Ste12 contributes to signal attenuation and the specificity of developmental fate*. *Eukaryot Cell*, 2006. **5**(12): p. 2147-60.