

# Synthetic Regulatory Circuits for Dynamic Control of Metabolism

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Recent research has significantly increased the known regulatory roles of RNA. Riboswitches are naturally-occurring RNA elements that control biosynthetic pathways. These elements occur within mRNA, directly bind metabolites, and regulate expression of the associated gene in response to changing metabolite levels<sup>1</sup>. Ligand recognition is mediated by sequences within the riboswitch referred to as aptamers, which form specific binding pockets. Aptamers to a target can be generated *de novo* and are capable of discriminating between structurally similar ligands<sup>2</sup>.

Synthetic riboswitches<sup>3</sup>, combining aptamer domains with an actuator such as a ribozyme, will provide modular biosensors which can then be integrated with cellular pathways. Riboswitches which respond to pathway intermediates or products offer the means to select for improvements in useful biosynthetic pathways. Once the desired pathway is in place, the same switches can be used to design and implement flexible control loops within the pathway.

Previous efforts with riboswitch selections have only been used to select for changes within the switch<sup>4</sup> rather than using an existing switch to select for new enzymes. An enzyme from the BM3 (CYP102A1) family of cytochrome P450 monooxygenases has been developed which produces sufficient theophylline to activate a theophylline-dependent riboswitch. The riboswitch can then be used *in vivo* to select for further improvements in enzyme activity.

Metabolic control systems, meanwhile, have generally focused on open-loop control of gene expression. While dynamic control has been demonstrated<sup>5</sup>, the particular system is not easily adapted to other pathways. Dynamic control using riboswitches that respond to key metabolites can improve productivity by reducing disturbances<sup>6</sup> and optimizing enzyme levels<sup>7</sup>. The theophylline-producing enzyme will be placed under negative autoregulation by a theophylline-responsive riboswitch. This feedback-controlled system will be grown in continuous culture and assayed for robustness of performance in the presence of disturbances.

1. Winkler, W.C., Nahvi, A., Roth, A., Collins, J.A. & Breaker, R.R. Control of gene expression by a natural metabolite-responsive ribozyme. *Nature* **428**, 281-286 (2004).
2. Jenison, R.D., Gill, S.C., Pardi, A. & Polisky, B. High-resolution molecular discrimination by RNA. *Science* **263**, 1425-1429 (1994).
3. Win, M.N. & Smolke, C.D. A modular and extensible RNA-based gene-regulatory platform for engineering cellular function. *Proc Natl Acad Sci U S A* (2007).
4. Lynch, S.A., Desai, S.K., Sajja, H.K. & Gallivan, J.P. A high-throughput screen for synthetic riboswitches reveals mechanistic insights into their function. *Chemistry & Biology* **14**, 173-184 (2007).
5. Farmer, W.R. & Liao, J.C. Improving lycopene production in *Escherichia coli* by engineering metabolic control. *Nature Biotechnology* **18**, 533-537 (2000).
6. Becskei, A. & Serrano, L. Engineering stability in gene networks by autoregulation. *Nature* **405**, 590-593 (2000).
7. Zaslaver, A. et al. Just-in-time transcription program in metabolic pathways. *Nature Genetics* **36**, 486-491 (2004).