GENE REGULATION

Switching genes off—all the way

A modular genetic switch that tightly regulates gene expression in mammalian cells will be useful in the design of complex synthetic gene circuits.

One of the goals of synthetic biology is the construction of molecular circuits from genetic modules. A requirement for successful engineering of such systems is very tight control over the genetic elements. In simple organisms such as bacteria and yeast, scientists have successfully designed tight genetic switches. The challenge was to transport this principle to mammalian cells.

James Collins from Boston University previously developed a toggle switch in bacteria based on two co-repressive genes: activity of gene *A* turns off the activity of gene *B* and vice versa. But when his team tried to implement this switch in mammalian cells, the system failed. He explains the reason: "The options available in mammalian systems to repress or inhibit gene expression were not strong enough. We needed to have a very tight off switch; 80–90% [inhibition] was not enough."

Inhibitors such as the tetracycline (tet) repressor or small interfering RNAs have a certain degree of leakiness, which make them unsuitable as sole regulators for tight switches. So Collins and his graduate student Tara Deans decided to follow the 'divide and conquer' paradigm; they combined repression at a lactose operon (*lac*) and a tet-controlled promoter with RNA interference as a means to get tight inhibition.

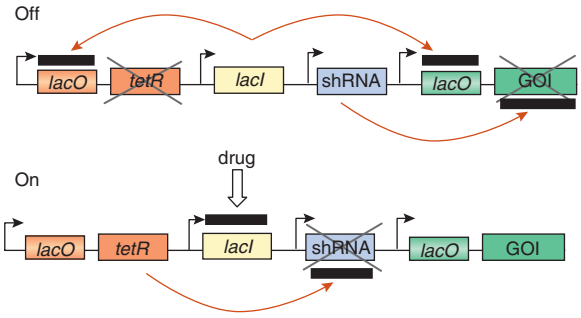

Figure 1 | A synthetic gene network. In the off state the Lac repressor is expressed and inhibits the Tet repressor (tetR) as well as the gene of interest (GOI). shRNA prevents any residual gene expression. In the on state, a drug inhibits *lacI* expression, resulting in expression of the GOI and the Tet repressor, which inhibits the shRNA.

Their expression cassette consists of four modules, each driven by a promoter under the control of either a drug or the gene product of an upstream module (**Fig. 1**). In the 'off' state, the Lac inhibitor represses the *lac* operon, which prevents expression of the Tet repressor and the target gene. Additionally, a short hairpin RNA (shRNA) against the target gene is expressed from a tet-respon-

METABOLOMICS

PUTTING A TRACE ON METABOLITES

Two independent groups describe isotopic labeling methods for detecting small-molecule metabolites for different applications.

Although it is perhaps less familiar than the genome or proteome—but arguably no less important—the metabolome is the total set of small-molecule metabolites in a biological system. Though metabolite profiling methods have actually existed for several decades, with the advent of systems biology there is a renewed interest in determining the roles of small molecules in complex signaling networks.

The detection and identification of small molecules in a biological system comes with a unique set of challenges. The metabolome consists of a great diversity of chemical structures, varying widely in their physical properties. Mass spectrometry and NMR spectroscopy have emerged as powerful tools for analyzing the metabolome: mass spectrometry is the more sensitive method, but NMR spectroscopy allows simultaneous measurement of all metabolites in a sample.

This unique ability of NMR spectroscopy to simultaneously detect all metabolites is also its stumbling block, resulting in acute peak overlap in the NMR spectrum, and making it extremely difficult to identify the components of a mixture. Daniel Raftery of Purdue University and his colleagues have come up with a simple yet powerful approach to identify classes of metabolites by using ^{13}C tagging (Shanaiah *et al.*, 2007).

The ^{13}C isotope is found in very low abundance in nature, so molecules in a mixture that are labeled with ^{13}C will give much stronger NMR signals. Raftery and colleagues used [1,1′-$^{13}C_2$] acetic anhydride to specifically and efficiently label amino acids in urine and serum samples. Using a two-dimensional NMR experiment called heteronuclear single quantum coherence (HSQC), they were able to quantitatively identify the labeled amino acids. Though a proof-of-principle study, this concept could be extended to ease the detection and quantification of many other metabolite classes, and should find use in systems biology applications as well as a rapid diagnostic screening method.

Another experimental challenge in understanding the metabolome is that it is rapidly and constantly changing. "To understand metabolic regulation, we need to understand how those fluxes and concentrations of metabolites change over time," says Joshua Rabinowitz of Princeton University, who has been interested in developing tools for understanding metabolism and its regulation at the systems level. In a paper last year, Rabinowitz and colleagues reported an approach called kinetic flux profiling (KFP), using mass spectrometry to trace the incorporation of an isotope-labeled nutrient into downstream metabolic products (Yuan *et al.*, 2006). Key to the method was the ability to rapidly swap unlabeled nutrient for labeled nutrient, which the researchers solved by growing their *Escherichia coli* on filters on top of the medium. Using mass spectrometry, they could monitor the isotope labeling pattern

sive promoter and removes any residual target-gene activity. To turn the switch on, a drug is added that inhibits expression of the Lac inhibitor, thus allowing expression of the target gene and the Tet repressor, which is needed to shut off shRNA expression from the tet-responsive promoter.

Collins and Dean showed that this intricate system relying on cross-talk between the different modules does indeed tightly regulate gene expression in mammalian cells. They used diphtheria toxin to prove that in the off state there was no detectable gene expression; otherwise, the cells—sensitive to even small amounts of the toxin—would not have survived. The cells only died once the researchers flipped the switch on, resulting in the expression of the diphtheria toxin.

Other useful features of this genetic circuit are that it can be used with any gene of interest and expression can be fine-tuned as desired.

Collins anticipates applications of the system in functional genomics, as well as for the study of normal development or disease onset and progression. He says, "Starting from a very tight off state you can explore phenotypic responses to titrated levels of gene expression, to establish threshold responses."

And of course it will be useful in synthetic biology. A well-characterized toggle switch can be incorporated into much more complicated circuit designs, which are needed to recapitulate pathways in mammalian cells.

Nicole Rusk

RESEARCH PAPERS

Deans, T.L. *et al.* A tunable genetic switch based on RNAi and repressor proteins for regulating gene expression in mammalian cells. *Cell* **130**, 363–372 (2007).

as well as the speed of incorporation, which provides insight into metabolic dynamics.

In a recent paper, Rabinowitz and his graduate student Jie Yuan took the KFP method a step further "to be able to figure out when you have branch points in metabolism, and which branch is being used," explains Rabinowitz. More specifically, they showed that KFP can distinguish between metabolites resulting from macromolecular decomposition versus those that are synthesized *de novo* (Yuan and Rabinowitz, 2007). Using the rapid isotope switching method with [^{15}N]ammonia as the label source, they added a carbon starvation step by transferring the cells on the filter from medium containing glucose to medium lacking glucose (or vice versa). *De novo*–synthesized metabolites incorporate ^{15}N, whereas those produced by macromolecular decomposition remain unlabeled. The researchers thus observed that under glucose starvation conditions cells switch from producing amino acids by synthesis to producing them by protein degradation.

Whether in NMR or mass spectrometry, systems biology or medical diagnostics, isotope labeling provides researchers with a powerful way to trace small-molecule metabolites.

Allison Doerr

RESEARCH PAPERS

Shanaiah, N. *et al.* Class selection of amino acid metabolites in body fluids using chemical derivatization and their enhanced ^{13}C NMR. *Proc. Natl. Acad. Sci. USA* **104**, 11540–11544 (2007).

Yuan, J. *et al.* Kinetic flux profiling of nitrogen assimilation in *Escherichia coli*. *Nat. Chem. Biol.* **2**, 529–530 (2006).

Yuan, J. & Rabinowitz, J.D. Differentiating metabolites formed from *de novo* synthesis versus macromolecular decomposition. *J. Am. Chem. Soc.* **129**, 9294–9295 (2007).

NEWS IN BRIEF

RNA INTERFERENCE

MicroRNA matchmaking

Computational microRNA target prediction programs usually yield a large number of targets for each microRNA, and deciding which to choose for validation is difficult. Grimson *et al.* describe five new features that determine the pairing of a microRNA to its target. These features are incorporated into their target-discovery algorithm, TargetScan, and will provide a more stringent selection for microRNA-target pairs.

Grimson, A. *et al. Mol. Cell* **27**, 91–105 (2007).

MICROSCOPY

Target-locking microscopy

Several microscope systems can target-lock an object and image it as it moves randomly in three dimensions. But these methods generally treat the object as a point, which makes the tracking of large complex objects such as cells difficult. Lu *et al.* describe target-locking acquisition with realtime confocal (TARC) microscopy that can follow a cell moving in three dimensions even as it changes shape, size and orientation.

Lu, P.J. *et al. Optics Express* **15**, 8702–8712 (2007).

GENOMICS

Mapping an abundance of SNPs

By resequencing the genomes of 15 different laboratory mouse strains, including 11 classical and 4 wild-derived ones, Frazer *et al.* identified more than 8 million unique single-nucleotide polymorphisms (SNPs) across the mouse genome with oligonucleotide arrays. They used the data to generate an ancestral haplotype map, available online (http://mouse.perlegen.com).

Frazer, K.A. *et al. Nature*, published online 29 July 2007.

PURIFICATION AND SEPARATION

Assaying sisterly cohesion

Sister chromatid cohesion is an essential process during cell division, requiring a multiprotein complex called cohesin. Ivanov and Nasmyth developed an *in vitro* system based on sucrose gradient centrifugation and gel electrophoresis, which allowed them to directly show that the cohesin ring in yeast affects sister chromatid cohesion by trapping the sisters within its ring.

Ivanov, D. & Nasmyth, K. *Mol. Cell* **27**, 300–310 (2007).

BIOSENSORS

Artificial tongues made from synthetic pores

There has been considerable interest in developing 'electronic tongues' for understanding the biology of taste and for developing sensing applications. Litvinchuk *et al.* report a universal stimulus-responsive pore to detect flavor molecules by using the concept of 'reactive amplifiers': molecules that react with analytes to enhance their pore-blocking ability and thus amplify the pore response.

Litvinchuk, S. *et al. Nat. Mater.* **6**, 576–580 (2007).