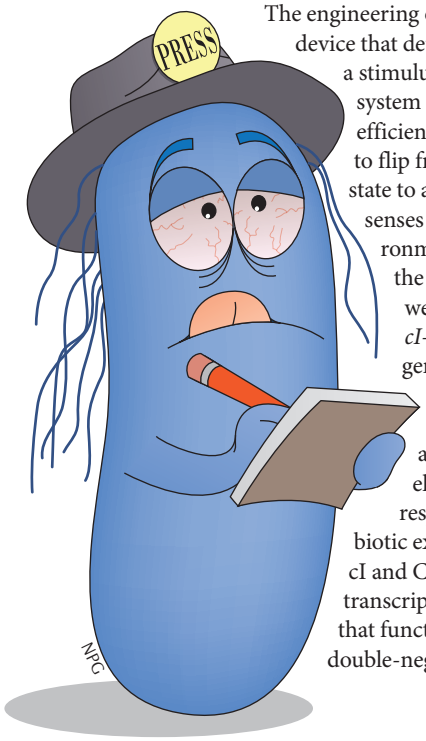
Nature Reviews Microbiology | AOP, published online 1 April 2014; doi:10.1038/nrmicro3256

SYNTHETIC BIOLOGY

E. coli reporter gets the inside scoop

> exposure to a low dose of anhydrotetracycline (ATC) causes the switch to flip



Recent progress in synthetic biology has enabled the construction of sophisticated genetic circuits to monitor environmental stimuli. Kotula *et al.* now engineer *Escherichia coli* with a genetic memory device that is capable of sensing, remembering and reporting on antibiotic exposure in the mammalian gut.

The engineering of a genetic device that detects and records a stimulus requires a system that can be efficiently triggered to flip from one stable state to another when it senses a specific environmental cue. Thus, the authors used the well-characterized *cI–cro* bistable genetic switch from bacteriophage λ to construct a memory element that responds to antibiotic exposure. The cI and Cro proteins are transcriptional repressors that function as part of a double-negative feedback loop in which cI represses *cro* expression and Cro represses *cI* expression. The cI state is extremely stable, so to generate an antibiotic-sensitive trigger for the Cro state, the *tetA* promoter was inserted upstream of *cro*, such that exposure to a low dose of anhydrotetracycline (ATC) causes the switch to flip and Cro to be expressed. The *cro* gene was fused to a *lacZ* reporter gene, which could be used as a readout to signal ATC exposure.

The entire circuit (the memory element and the trigger) was inserted into the chromosome of *E. coli* K12 (strain PAS132), and after less than 4 hours of ATC exposure *in vitro*, the switch had flipped to the Cro state, and it remained in this state for approximately 150 cell divisions. Importantly, growth competition experiments between wild-type *E. coli* K12 and strain PAS132 showed that the circuit had negligible fitness costs. The authors then went on to determine whether the system was functional in the complex environment of the mouse gut, and found that almost all of the PAS132 cells isolated from the faeces of mice that were treated with ATC had switched to the Cro state within 1 day and that >50% of the population remained in this state for more than 1 week after the withdrawal of ATC. By contrast, PAS132 cells isolated from untreated control mice did not switch and remained in the cI state.

As PAS132 is a laboratory-adapted strain, it was gradually outcompeted in the mouse gut by the endogenous microbiota. Thus, the authors transferred the system into a native *E. coli* isolate (strain PAS133) of the gut and found that the sensing and memory functions were essentially identical to those in PAS132; moreover, PAS133 was capable of stably colonizing the gut for longer than PAS132.

These data highlight the power of synthetic biology to harness microorganisms in the engineering of detection and diagnostic tools to monitor stimuli in complex and ill-defined environmental niches, and provide proof-of-principle of the potential to exploit naturally designed circuits, such as the *cI–cro* switch, for this purpose.

Christina Tobin Kåhrström

ORIGINAL RESEARCH PAPER Kotula, J. W. *et al.* Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1321321111 (2014)