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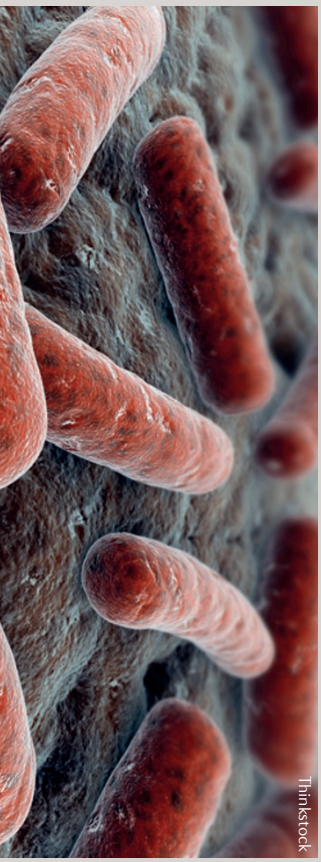
MICROBIAL GENETICS

Bacterial sensors report on the gut



The mammalian gut contains a wide variety of microbial flora and fauna, the disruption of which can lead to a number of disorders, including inflammatory bowel disease and autoimmune disease. However, technologies to determine changes in the gut microbial environment are limited. Now, Kotula *et al.* have generated a new tool with potential applications to this problem: bacteria that are capable of surviving in the murine gut and that can sense, 'remember' and 'report' on their 'experiences'.

In order to engineer bacteria that have cellular memory (that is, cells with a sustained cellular response in the presence of a transient stimulus), the authors used a genetic switch. Rather than engineering a novel one, they modified the naturally occurring *cI*/*cro* genetic switch from bacteriophage λ, which is "one of the best bacterially characterized genetic transcriptional circuits known", according to Pamela Silver (Harvard Medical School), the senior author of the study. The *cI*/*cro* switch is a mutually repressive genetic switch, such that expression of *cro* represses expression of *cI* and vice versa, resulting in two stable genetic states. The authors engineered their construct to make the *cro* state inducible by incorporating a *tetA* repressor–promoter segment 'trigger' upstream of the *cro* gene. In the presence of low doses of androtetracycline (ATC), the *tetA* repressor will be inhibited, which allows the *tetA* promoter to drive expression of *cro*, whereas in the absence of ATC, the *cI* state will prevail. To have a readout of *cro* expression, a *lacZ* reporter was used that turns bacteria in the *cro* state blue in the presence of X-gal. Putative 'memory elements' were inserted downstream of the *cro* gene; more than ten such elements were tested and four were characterized in further detail. Finally, Kotula *et al.* used recombineering so that the entire construct is incorporated into the genome of the commonly used K12 strain of *Escherichia coli* and is thus inherited stably.

The team tested their constructs *in vitro* and found that *E. coli* exposed to ATC stably switched to the *cro* state, and that bacteria containing the memory element remained in this state for five days following removal of ATC. Neither the triggering nor memory elements had an effect on bacterial survival or growth.

The authors then tested their bacteria *in vivo* by administering the bacteria to mice. They first cleared the gut of other flora by streptomycin treatment and then administered their bacteria in the presence and absence of ATC. As in the *in vitro* experiments, the bacteria maintained the *cro* state for more than a week even when ATC was removed. Endogenous gut bacteria began to recolonize as soon as streptomycin treatment ended and outcompeted the inserted bacteria after eight days, which limited the time that the artificial bacteria could be used. To solve this problem, the authors isolated an uncharacterized form of *E. coli* that is endogenous to mice and inserted their construct in this bacterium. Similar to the K12 *E. coli*, this engineered strain (PAS132) could sense, record and remember exposure to ATC. Importantly, PAS132 populations eventually stabilized to around the same numbers as other natural gut flora. This suggests that laboratory strains can be used to engineer constructs that can then be inserted into more biologically feasible strains for use *in vivo*. "This may be the first instance of a robust synthetic reporter system that can report on something relevant to the physiology of the animal," says Silver.

The potential applications of this work for health and disease are immense, and Silver and her group now plan to make further memory devices engineered to register other gut environment sensors, such as diet or inflammation.

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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)