MICROBIOLOGY

Better tools for *Bacteroides*

Two sets of complementary tools extend our capacity to manipulate the gut microbiome.

Escherichia coli may be the most famous gut denizen, but the most abundant residents of the Western gut are members of the *Bacteroides*, a genus with a meager genetic toolbox that reflects its B-list status. Two recent studies raise the profile of these important microbes by engineering tools to track strains in space and time and to alter gene expression *in vivo*. These new tools allow precise perturbations of the microbiome as well as highly resolved mechanistic studies of host–microbe interactions and suggest the use of *Bacteroides* as vectors for microbe-mediated therapeutics.

Justin Sonnenburg and his team at Stanford University work on gut–bacteria interactions, a subject that attracted Weston Whitaker. "We had a number of ideas for projects to apply my synthetic biology background," says the postdoc. "All of them involved expressing protein." After exhaustive testing, Whitaker was dismayed to find that existing tools could not express GFP at levels high enough for imaging of *Bacteroides*.

To develop useful expression tools, he first designed high-throughput cloning and gene transfer workflows that shaved time off of standard protocols. Then, using promoter conservation analysis, ribosome-binding site library screening and systematic promoter mutagenesis, Whitaker was able to generate a series of phage-based constitutive promoters that function predictably over a million-fold expression range. At levels beyond the range of endogenous expression, he achieved bright GFP and RFP fluorescence without affecting bacterial fitness.

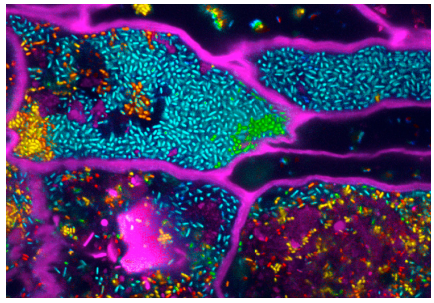
The researchers utilized different GFP and RFP levels to uniquely mark six *Bacteroides* strains and study their distribution in the colon of inoculated mice. The researchers could directly show that an established strain prevents a second isogenic strain from colonizing the colonic crypts and, to a lesser extent, the lumen. Whitaker notes that fluorescence *in situ* hybridization (FISH) can also be used to image strain localization but is more useful when spanning a wide phylogenetic range; it is more challenging to separate closely related strains.

Better expression tools had also been on the long-time wish list of Andy Goodman and his group at Yale University. Goodman wanted to address two gaps in existing *Bacteroides* tools, timing—"when is a gene required, and how long does its function or its effect persist in the host?"—and functional dosing, "the question of tuning the expression levels of a microbiome-encoded function to look at dose response."

Inducible promoters do exist for *Bacteroides*, but they are derived from native, sugar-responsive promoters. To establish a biorthogonal system, the researchers rebuilt the Tet repressor system from *E. coli* for *Bacteroides thetaiotaomicron*. The Tet operator is repressed by a constitutively expressed Tet repressor, and this repression is relieved by adding anhydrotetracycline. Promoter alignments across 180 *Bacteroides* genomes identified less conserved regions that might be more permissive to the insertion or substitution of the operator sequence.

After trying a large number of promoter configurations and ribosome binding site sequences, the researchers found promoters that do not leak in the absence of inducer, are dose responsive and can be induced over four orders of magnitude. They then used these promoters to study changes in levels of sialic acid, a sugar that decorates host proteins in the gut epithelial lining and is metabolized by some bacteria. Sialidase activity in commensal bacteria can liberate sialic acid, which the Sonnenburg group originally showed is an important factor in pathogen colonization, especially after antibiotic treatment.

By engineering inducible sialidase in microbes that do not consume sialic acid, Goodman's team discovered that sialic acid persists in the gut after induction ends. They could thus measure the 'legacy' of microbiome function to quantify its persistence, says Goodman. They also discovered that sialidase is present in excess in the gut, and the reaction is substrate limited—a surprising result, since microbial functions are often thought to be directly related to enzyme copy number. Goodman posits that after antibiotic treatment, a drop in bacterial mass lowers sialic acid consumption, which raises the levels available for pathogens.



New genetic tools can label different *Bacteroides* strains in the gut. Reprinted with permission from Whitaker *et al.* (2017).

Looking ahead, Whitaker is interested in engineering systems that can record what bacteria sense using logic conditions, and Goodman is looking to analyze old questions along the time and dose-response dimensions. The two approaches are complementary; "you could certainly imagine ways of connecting these two projects together," says Goodman. Whitaker also notes that as an important commensal, "Bacteroides would be a natural place to go if you wanted to engineer a cell to deliver therapeutics to the gut."

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RESEARCH ARTICLES

Lim, B. *et al.* Engineered regulatory systems modulate gene expression of human commensals in the gut. *Cell* **169**, 547–558.e15 (2017).

Whitaker, W.R. *et al.* Tunable expression tools enable single-cell strain distinction in the gut microbiome. *Cell* **169**, 538–546.e12 (2017).