Scientists invent new tool for the synthetic biologist's toolbox

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Researchers at the University of California San Diego have invented a new method for controlling gene expression across bacterial colonies. The method involves engineering dynamic DNA copy number changes in a synchronized fashion. The results were published in the July 10, 2017 online edition of *Nature Genetics*.

Until now, methods for controlling or programming bacterial cells

involved transcriptional and post-transcriptional regulation. UC San Diego researchers led by Jeff Hasty, a professor of bioengineering and biology and member of the UC San Diego Center for Microbiome Innovation, describe a new method, which involves cutting circular pieces of bacterial DNA called plasmids, effectively destroying the DNA and turning off regulation.

The study also demonstrates how DNA concentration can be increased to turn on a synthetic gene circuit. By controlling DNA copy number, researchers can effectively regulate gene expression.

Synthetic biology - which can involve altering biological systems for some purpose - is emerging as an engineering discipline. The field was firmly established in 2000, with the description of synthetic biological circuits in which parts of a cell are designed to perform functions, similar to the way an electronic circuit works. Also similar to an electronic circuit, the task performed by a biological circuit can be turned on and off. At the same time, researchers described the making of a "genetic clock," which involves placing genes in a particular order so that they'll be turned on at a specific time. This approach has also helped researchers understand natural "oscillators," such as our sleep-wake cycle.

Since these early inventions, Hasty and his team have shown how engineered cellular oscillations can be synchronized within a bacterial colony using plasmids, synthetically designed by the researchers themselves. Now, the team is adding a new tool to the Synthetic Biologist's toolbox - a "master clock" of sorts that will allow researchers to coordinate subprocesses in bacterial cells.

"This remarkable achievement is a key building block for controlling microbiomes", said Rob Knight, professor of pediatrics at UC San Diego with a joint appointment in computer science and engineering. Knight

leads the Center for Microbiome Innovation. "By controlling different strains with the same master clock, or by giving different strains their own clocks, we can start to engineer population-level dynamics to control specific microbiome functions."

Examples of these functions might include interaction with host cells at particular times of day, such as timed release of neurotransmitters produced by the bacteria, or interactions with other bacteria such as antifungal production triggered by a meal rich in sugar.

Programming the clock

The researchers used an endonuclease from Saccharomyces cerevisiae, a species of yeast, expressed alongside a plasmid containing the nuclease recognition sequence to temporarily reduce the plasmid's copy number below natural levels.

"We found that plasmid replication is so strong that we couldn't cut them all," said Hasty. "This was good news, because it meant we could down-regulate gene expression, but not eliminate it."

The researchers reasoned that the method could be used to regulate an entire suite of genes and promoters, and tested their idea using a previously constructed circuit to produce sustained cycling of DNA plasmid concentration across a colony of *E. coli* cells.

The circuit works by using a small molecule, known as AHL, to coordinate gene expression across a colony of bacterial cells. Once on, the genes driven by the promoter are also activated, including the AHL-producing gene itself. Thanks to this positive feedback loop, the more AHL accumulates, the more it is produced. Because AHL is small enough to diffuse between cells and turn on the promoter in neighboring cells, the genes activated by it would also be produced in high amounts,

leading to a phenomenon known as quorum sensing. Hasty and his team employed the endonuclease to reduce the number of these plasmids present in the colony and used this mechanism as negative feedback to driving the oscillations in gene expression. Using quorum sensing, the feedback system was coupled across the colony of cells.

"We observed regular oscillations of gene expression in microfluidic chambers at different colony length scales and over extended time periods," said Hasty. "By incorporating elements for both positive and negative copy number regulation, we were able to improve the robustness of the circuit."

More information: Synchronized DNA cycling across a bacterial population, *Nature Genetics* (2017). DOI: 10.1038/ng.3915

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