Biologists create toolkit for tuning genetic circuits

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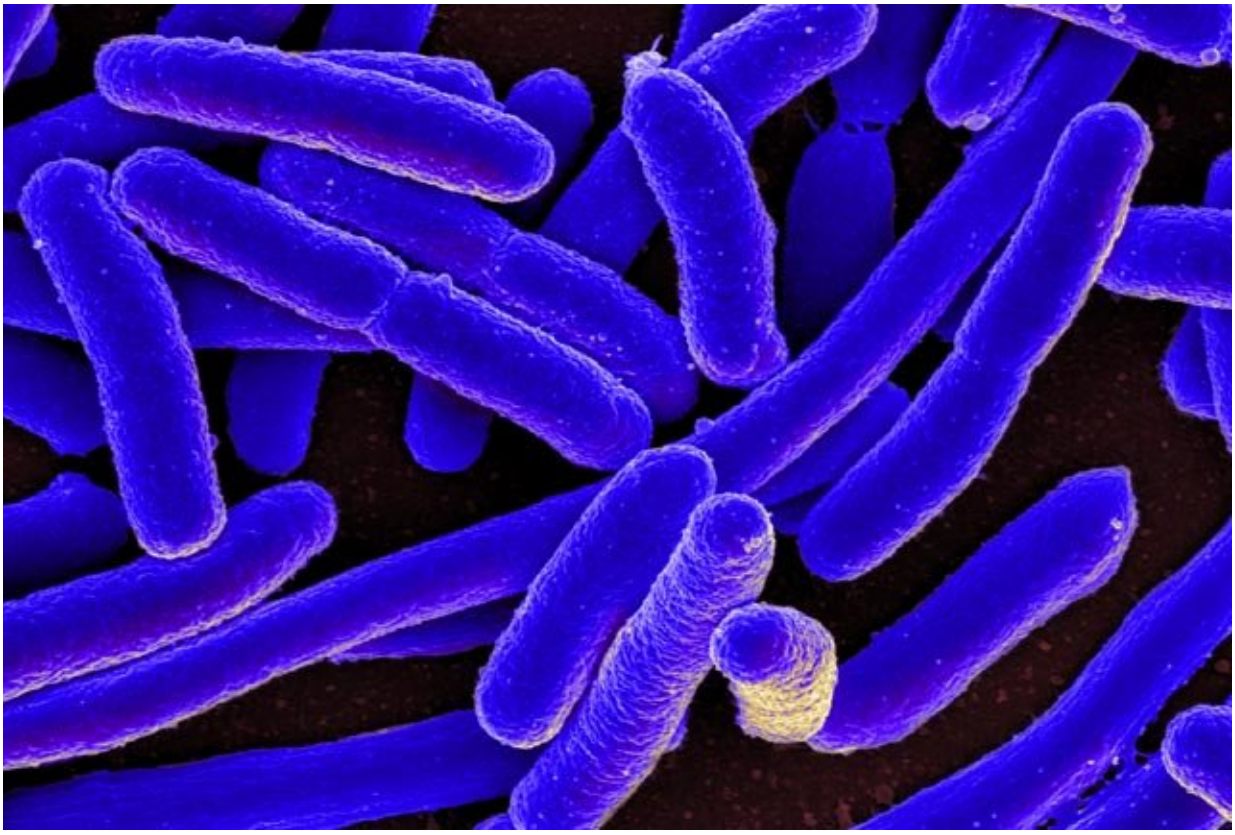

Escherichia coli. Credit: NIAID

Rice University scientists have created a toolkit for synthetic biologists who need to precisely tune the input and output levels of genetic circuits.

The research, which is online in Nature Communications, is a boon for life scientists who systematically engineer bacteria and other organisms to perform tasks they wouldn't naturally do.

"Probiotics are one example," said Matthew Bennett, associate professor of biosciences at Rice and co-lead scientist of the new study. "They're beneficial gut bacteria that are essential for human health, and many synthetic biologists are looking at ways to design probiotics that can diagnose or fight disease. Such engineered probiotics would be able to produce drugs or other complex molecules within the human body to fight diseases ranging from cancer to inflammatory bowel disease."

Producing drugs when and where they are needed in the body would open new doors for fighting disease, but, Bennett said, synthetic biologists have struggled to design circuits that are precise enough for drug delivery.

"Synthetic biologists need to create genes that turn on or off in response to environmental cues," he said. "These act as sensors, allowing the probiotic to produce the drug when it is needed based on environmental cues."

Using the bacteria Escherichia coli, Bennett, graduate student Ye Chen, postdoctoral researchers Joanne Ho and David Shis and colleagues from the University of Houston, employed modular molecular building blocks to create promoters that turn genes on and off as much as needed.

While genetic circuits are like electric circuits in some ways, the switches for turning them on and off are far more complicated. By themselves, genes cannot make the proteins they encode. Instead, specialized enzymes read the genes and stamp out proteins based on what they read. Gene promoters are another specialist in this process.

"A promoter drives a gene," Bennett said. "It initiates the decoding and determines when the gene is turned on or off.

"Synthetic biologists have been engineering promoter regions to respond to different chemical cues, but we have been stuck with what nature has given us," he said. "A naturally occurring promoter that responds to a chemical might not behave well when used in a synthetic gene circuit. It might not turn the target gene on or off as much as we would like. If a bacterium wants to sense a particular chemical cue, it will turn a gene on or off in the way it needs to. It might turn it on a little bit, or it might turn it on a lot. We did not have much control over that before."

In addition, Bennett said, many promoters are "leaky" in the sense that even when they switch a gene off it still produces small amounts of protein.

"There are evolutionary reasons why leakiness can arise in nature, but when you're engineering a circuit, you need more precision," he said.

Promoters are regions of DNA that are part address line and part instruction manual. They not only tell transcription proteins where to start reading a gene, but they also regulate how strongly the gene is turned on—whether it produces a lot or a little protein. Using a modular approach, Bennett's team developed a design scheme for creating nonleaky promoters that turn on as much as needed.

University of Houston mathematicians Krešimir Josić, Chinmaya Gupta and William Ott calculated some of the specific properties that would be needed for each building block and worked with Rice team members who designed, created and tested them in E. coli. Various blocks were mixed and matched to form a library of promoters, each of which was designed to react in a specific way to one or more chemical inputs.

For example, in a genetic circuit, one gene can be programmed to switch on when it receives a specific cue, and the product of that gene can be a small molecule protein that in turn activates or turns off another gene. By stringing together whole sets of these genes, synthetic biologists can build complex circuits.

"This ability is key for constructing synthetic gene regulatory circuits that require precise input and output relationships," Bennett and colleagues wrote in their Nature Communications paper. "This paper provides a simple, cost-effective means of engineering promoters that provide user-defined dynamic ranges, which will enable the fine-tuning of the metabolic flux within synthetic biological and chemical circuits inside living cells."

Bennett said another key element of the project was designing promoters that could be activated only in the presence of two or more cues.

"Nature gives us only a few examples of promoters that use multiple inputs, so designing nonleaky, easy-to-use multi-input promoters was a high priority for us," he said.

More information: Ye Chen et al. Tuning the dynamic range of bacterial promoters regulated by ligand-inducible transcription factors, *Nature Communications* (2017). DOI: 10.1038/s41467-017-02473-5

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