MICROBIOLOGY

Recording bacterial responses to changes in the gut environment

A CRISPR-based tool reveals intestinal microbiota gene expression through time

By **Liron Zahavi**[1,2] and **Eran Segal**[1,2]

The bacterial population in the human gut is tightly linked with host health and has been implicated in a wide array of diseases, such as atherosclerosis, diabetes, and cancer (*1*, *2*). Existing treatments that are intended to shape the microbiota to improve health—prebiotics, probiotics, and fecal microbiota transplantations (FMTs)—have limited success in obtaining the desired microbial composition and in maintaining it over time (*3*, *4*). This is largely because bacteria in the gut are affected by other bacteria, human physiology, diet, medications, and more, and the relationships in this complex system are yet to be deciphered. Understanding the bacterial response and adaptation to these variables is essential for creating more effective microbiota-based interventions. On page 714 of this issue, Schmidt *et al.* (*5*) present a new tool that "documents" bacterial gene expression in mice and can clarify the bacterial response to perturbations in the intestinal environment.

Most studies that investigate the effect of different factors on the gut microbiome are based on DNA profiling of fecal samples. Such studies compare the DNA content of microbiomes and associate differences with environmental perturbations. This approach reveals changes in species composition or in bacterial genomics, reflecting the microbiome response on ecological and evolutionary time scales, respectively. However, the most immediate bacterial response happens at a physiological time scale, which is not reflected in DNA (*6*). One immediate means of adaptation to environmental changes is gene expression. Thus, RNA-based studies fill an important gap for understanding bacterial adaptation to host variables.

Schmidt *et al.* introduce a method for studying microbial transcription. They demonstrate this method by colonizing mice with an engineered *Escherichia coli* strain, inserting the Record-seq system, which leverages the recording nature of CRISPR-Cas (*7*). Within the Record-seq–engineered *E. coli* cells, transcripts are reverse transcribed to DNA and stored as spacers in a CRISPR array. As transcriptional records are stored as DNA sequences in the CRISPR array, bacterial gene expression history can then be sampled by DNA sequencing of host fecal samples (see the figure). They use this recording tool to investigate mechanisms of *E. coli* adaptation to varying host diets, cocolonization with another species, bacterial gene deletion, and host inflammation.

They uncovered, for example, multiple metabolic genes whose expression was increased when the mice were fed a less diverse diet. They hypothesized that these genes allow the bacteria to feed on host mucosal sugars and predicted that a bacterium lacking two of these genes will have a competitive disadvantage when the host is fed with a restricted diet, which they validate experimentally. This shows the potential of this method to highlight genes that allow bacteria to adapt to specific host conditions. These insights could be used to design an intervention that is based on bacteria that fit the host diet or to adjust host diet to allow certain bacteria to thrive. Similarly, they demonstrate the use of Record-seq to analyze the effect of introducing another species on *E. coli* gene expression and deduce that the bacteria shift their metabolism to exploit new niches created by the other species. Such experiments can be informative about bacteria-bacteria interactions in a mechanistic resolution that species co-occurrence measurements cannot.

The ability to look at microbial gene expression is not new. RNA-sequencing (RNA-seq)–based metatranscriptomics is a common tool with which to investigate the microbiome (*8*). There are, however, considerable advantages to Record-seq over RNA-seq. Although RNA-seq shows the immediate response of gut bacteria to its environment, it only reflects a snapshot of the microbiome at the moment of the sample. Because of the short lifetime of mRNA molecules, samples need to be taken at the exact moment of the response to investigate transient states. Record-seq, however, is distinctive in that it archives transcripts in the engineered *E. coli* DNA over time and thus describes a series of states. When Schmidt *et al.* changed the diets fed to mice, Record-seq identified signs of the previous diet after more than a week, whereas the signal was rapidly lost with RNA-seq analysis of fecal samples.

In a different experiment, Schmidt *et al.* demonstrate another advantage of their method: the ability to attribute transcripts to a specific CRISPR array and, therefore, bacterial strain. In metatranscriptomics, RNA is extracted from fecal samples and represents the wide array of species colonizing the gut. Attributing mRNA molecules to genomes depends on the specificity of the short mRNA sequence to a single genome. Schmidt *et al.* exemplify the importance of this limitation. They barcoded two CRISPR arrays and transformed the recording plasmids into *E. coli* cells of two strains. One was a wild-type strain, and the other had one gene deleted. They cocolonized the two strains in mice and studied the effect of the deletion

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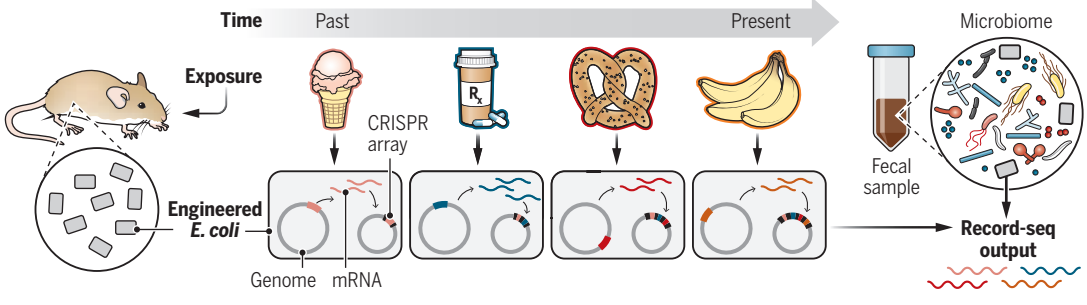
GRAPHIC: KELLIE HOLOSKI/*SCIENCE*

Recording microbial responses in vivo

Schmidt *et al*. engineered *Escherichia coli* cells to record their gene expression in the mouse intestine. Within the engineered cells, the Record-seq system incorporates reverse-transcribed transcripts as new spacers in a barcoded CRISPR array on plasmids. Sequencing mice fecal samples revealed the bacterial response to ongoing and past exposures, such as diet changes and intestinal inflammation.



on gene expression of that strain compared with the wild type, showing the dynamics between the two similar strains in vivo. This analysis could not have been done with metatranscriptomics.

The archive quality of Record-seq could be leveraged for clinical applications. In diabetes mellitus, following the patient's blood glucose concentration is important for assessing disease progression and the effectiveness of the treatment. Although blood glucose concentration indicates whether the patient is in hyperglycemia at the moment of sampling, there is a measure—percentage of glycated hemoglobin (%HbA1c)—that reflects the glycemic state of the patient over the past 3 months. This test is invaluable for the diagnosis and management of diabetes, exemplifying the importance of a single test that captures longitudinal information. Other chronic diseases whose management depends on lifestyle adjustments can benefit from such a test. Schmidt *et al.* show that Record-seq–engineered sentinel cells can identify the existence and severity of intestinal inflammation in a colitis mouse model. Celiac patients, for example, could benefit from such a system of bacterial sentinel cells that monitors their adherence to dietary restrictions and the state of their disease. Furthermore, if successfully adapted to be used in human microbiota, this system can be used to design an assay for diet effects on inflammatory bowel disease (IBD) to develop a personalized nutritional plan to minimize intestinal inflammation in patients (*9*).

The method that Schmidt *et al.* introduce opens new avenues for studying the microbiota response to environmental factors and paves the way toward the development of more effective microbiota-based treatments. Two of the major advantages of their method—its ability to "document" responses in changing states and to associate outputs with specific strains within a complex community—are very much missing from other microbiota -omics research methods. Proteomics- and metabolomics-based research methods, which cover other important layers in microbiota dynamics and its interactions with the host, would benefit from similar improvements. ■

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10.1126/science.abq1455

MATERIALS SCIENCE

Beating natural proteins at filtering water

Artificial fluorous channels outperform aquaporins in water permeation

By **Yuexiao Shen**

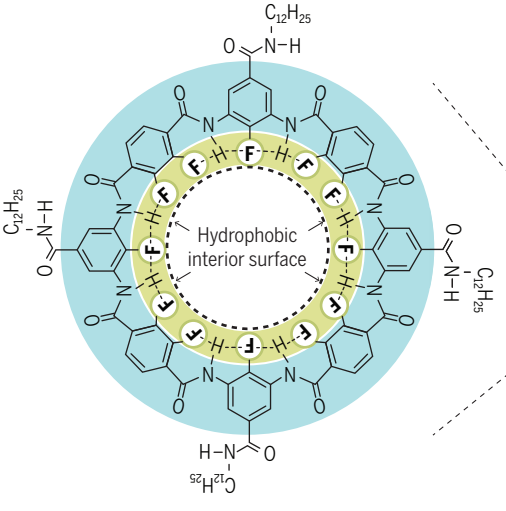
Humans have long drawn inspiration from nature to create tools that can mimic a specific function. In recent decades, researchers have taken this philosophy to the nanoscopic level, such as in the creation of synthetic structures for water purification inspired by biological systems. Aquaporins are a type of naturally occurring protein known as water channels, which have notable water permeability and selectivity (*1*). On page 738 of this issue, Itoh *et al.* (*2*) report the design of fluorous channels—structures made out of fluorocarbons—that can self-assemble from oligoamide nanorings and outperform biological water channels in permeability. Because of the superhydrophobic interior surface endowed by densely covered fluorine atoms, these nanochannels exhibit a water permeation flux that is two orders of magnitude greater than that of aquaporins.

Nature evolves ideal water-selective channels, including aquaporins, that are embedded in cell membranes to regulate osmotic balance and make critical biophysical contributions to living organisms. Aquaporins form an hourglass-shaped narrow channel with a ~0.3-nm aperture and a hydrophobic interior surface, resulting in notable water permeability and solute rejection (*1*). However, these energy-efficient structures have been difficult to mimic. The first artificial water channels could accommodate water molecules, but the mobility was slow and there was little rejection of other small molecules (*3*, *4*). Later synthetic structures maintained high water conductance (*5*–*8*) while also rejecting salts, including NaCl (*6*–*9*), and even protons (*7*, *8*).

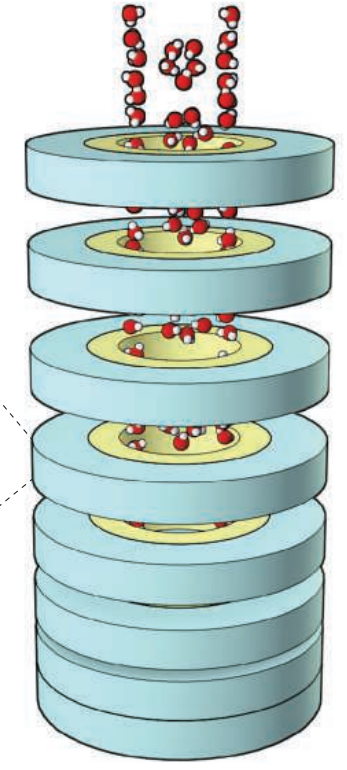
When water flow is confined to a subnanometer channel with hydrophobic interior surfaces, classical hydrodynamics is no longer applicable. The clustered arrangement of water molecules breaks

A frictionless water channel

Itoh *et al.* designed a nanochannel with a superhydrophobic interior surface that beats the naturally occurring protein aquaporin at its own game. The stacked nanorings form a nanochannel where water clusters are broken down into a single file as they flow through.



The ring is fabricated by condensing fluorine-containing amide monomers. The electronegative fluorine (F) atoms interact with the adjacent hydrogen atoms, which stabilizes the ring structure.



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Science, 376 (6594), • DOI: 10.1126/science.abq1455