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The bacterial population in the human gut is tightly linked with host health. The gut microbiome has been implicated in a wide array of diseases, such as atherosclerosis, diabetes, and cancer [1,2]. With the understanding of its importance for health, the effort and need to modify it grows too. Albeit this effort, existing treatments aimed to shape the microbiome — prebiotics, probiotics, and fecal microbiota transplantations (FMTs) — have very limited success in obtaining the desired microbiome composition and in maintaining it over time [3,4]. This is largely because bacteria in the gut are affected by other bacteria, by human physiology, diet, medications, and more, and we have yet to decipher the relations in this complex system. Understanding the bacterial response and adaptation to these variables is essential for creating more effective microbiome-based interventions, as it can help choose or engineer bacteria that are better fitted to a specific host or guide the adjustment of other variables for the bacteria to persist. On page XX of this issue, Schmidt *et al.* present a novel tool that "documents" bacterial gene expression *in vivo* and can shed light on the bacterial response to perturbations in the intestinal environment.

Most studies investigating the effect of different factors on the gut microbiome are DNA-based. 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 timescales, respectively. However, the most immediate bacterial response happens at a physiological timescale — which is not reflected in the DNA [5]. One immediate means of adaptation is gene expression, which changes to provide the bacterial needs in the new environment. Thus, RNA-based studies fill in an important gap for understanding bacterial adaptation to host variables.

In their paper, Schmidt *et al.* introduce a method for studying microbiome transcription. They demonstrate this method by colonizing mice with an engineered *E. coli* strain, inserting the CRISPR-based Record-seq system [6]. Record-seq incorporates bacterial transcripts back into the bacterial genome, enabling the sampling of bacterial gene expression history by sequencing host fecal samples. They use this recording tool to investigate mechanisms of bacterial adaptation to varying host diets, co-colonization with another species, 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 bacteria lacking two of these genes will have a competitive disadvantage when the host is fed with a restricted diet — which they validated 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 — deducing that it shifts its metabolism to exploit new niches created by the other species. Such experiments can teach about bacteria-bacteria interactions in a mechanistic resolution that species co-occurrence measurements cannot.

The ability to look at the microbiome gene expression is not new. RNA-seq based metatranscriptomics is a common tool to investigate the microbiome [7]. There are, however, significant advantages to Record-seq over RNA-seq. While RNA-seq shows the immediate response of the gut bacteria to its environment — it only reflects a snapshot of the microbiome at the moment of the sample. Due to the short lifetime of mRNA molecules, to investigate transient states, samples need to be taken at the exact moment of the response. Record-seq, however, is unique in that it archives transcripts into the bacterial genome over time, and thus describes a sequence of states. When Schmidt *et al.* changed mice diets, Record-seq identified signs of the previous diet after more than a week — while the signal was rapidly lost with the RNA-seq.

In a different experiment, Schmidt *et al.* demonstrated another advantage of their method — the ability to attribute transcripts to a specific CRISPR array, and therefore, genome. In metatranscriptomics, RNA is extracted from fecal samples and represents the wide array of species colonizing the microbiome. Associating mRNA molecules with genomes depends on the uniqueness of the short mRNA sequence to a single genome. Schmidt *et al.* exemplify the significance of this limitation. They barcoded two CRISPR arrays and inserted them into two *E. coli* genomes. One genome was of a wt strain, and the other had one gene deleted. They co-colonized the two strains in mice and studied the effect of the deletion on gene expression of that strain compared to the wt, showing the dynamics between the two similar strains *in vivo*. This analysis could not have been done based on metatranscriptomics.

The archive quality of Record-seq can be leveraged for clinical applications. In diabetes melitus, following the patient's blood glucose level is important for assessing the progression of the disease and the effectiveness of the treatment. While blood glucose level 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 three months. This test is invaluable for the diagnosis and management of diabetes. Other chronic diseases whose management depends on lifestyle adjustments can benefit from such a test. Schmidt *et al.* show that Record-seq can identify the existence and the severity of intestinal inflammation in a colitis mouse model. Celiac patients, for example, could benefit from such a system of sentinel cells for monitoring their adherence to the dietary restrictions and the state of their disease. Furthermore, if successfully adapted to be used in human microbiomes, this system can be used to design an assay for diet effect in inflammatory bowel disease (IBD) patients in order to develop a personalized nutritional plan to minimize intestinal inflammation [8].

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

References

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