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Dyeing to connect

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Abstract

Culture-independent methods capable of connecting bacteriophages (phages) to their target host bacteria will help define the roles of phages in host-associated microbiotas. A recent study used fluorescently dyed intestinal phages to identify novel phage–bacterial interactions from the human intestine.

Phages are viruses that infect bacteria. Phages constitute a major component of the human intestinal microbiota, yet the impact of phages on intestinal microbial community structure and human health is poorly understood. In this issue of *Nature Microbiology*, Džunková and colleagues use a high-throughput viral tagging method to explore phage–bacterial host interactions in the human intestine¹. Determining how phages influence intestinal biology and microbiota ecology is of great importance to human health. Phages modify bacterial communities via the molecular exchange of DNA (that is, transduction), which contributes to bacterial virulence and metabolism². The spontaneous induction of prophages from bacterial chromosomes can alter bacterial communities by killing competitors and increasing the competitive fitness of prophage-containing bacteria^{3,4}. Renewed interest in phage therapies, including their potential role in the efficacy of faecal microbiota transplantation (FMT), where stool (containing bacteria and viruses) from healthy donors is used to restore microbial diversity in intestines, could set the stage for novel phage therapeutics for the amelioration of a variety of intestinal diseases⁵.

Assigning identity to phages and their target host bacteria is a major barrier to understanding how phages influence human health. Traditionally, the identification of phage–host interactions has relied on either low-throughput cultivation techniques or in silico predictions based on a limited number of sequenced phage genomes or phage genetic signatures that can sometimes be ambiguous⁶. Džunková et al. aimed to characterize phage–bacteria interactions within the human intestinal microbiota using single-cell virus tagging⁷. Phages are first isolated from faecal filtrates and their nucleic acids stained with a fluorescent dye (Fig. 1). Labelled phages are then mixed with native intestinal bacteria and sorted via flow cytometry to isolate phage–bacteria binding partners. The phage-tagged bacterial cells and associated phages are sequenced, revealing the identity of the phage–bacterial host pairings. Using this approach, 363 novel phage–host bacterial pairings were

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Competing interests

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discovered, greatly expanding our current knowledge of phage–bacterial interaction networks in the intestine. They observed that the majority of phages from the intestine had exquisite specificity for interactions with a single bacterial host species (Fig. 1). This suggests that low bacterial host promiscuity may benefit future phage therapies designed to engineer the intestinal microbiota during health and disease.

There has been intense debate on whether phages are important contributors to the horizontal transfer of genes such as antibiotic resistance determinants within the intestinal microbiota⁸. In this study, Džunková and colleagues identified a minority subset of phages that were capable of tagging bacteria of different species. This finding suggests that phages may not be significant drivers of horizontal gene transfer (HGT) among bacteria outside of individual species. Other recent findings support the idea that HGT may be more specifically linked to other means of DNA mobilization, such as conjugation⁹.

Intestinal phage communities are considered to be compositionally stable but discordant between individuals¹⁰. The majority of phages identified in this study were generalists capable of tagging bacteria isolated from multiple distinct human subjects (Fig. 1). When phages from one subject were used to tag bacteria from unrelated subjects, they found that a large portion of bacteria in all subjects were targeted by phages from diverse donors. This suggests that the introduction of phages between patients, such as during an FMT, could result in phage-mediated bacterial dysbiosis in recipient patients. However, the viral-tagging method utilized in this study measures adsorption as a metric of phage–bacterial interaction and does not determine active phage infection. While adsorption may indicate phage–bacterial pairing, off-target effects, consisting of phage binding but lack of infection, are possible. Follow-up studies are needed to confirm that the observations made with this method indicate successful phage infection of bacterial hosts. Demonstrating infection would also strengthen the argument that cross-donor phages from an FMT could lead to phage-mediated bacterial dysbiosis in recipient patients.

This study brings us one step closer to more fully characterizing the complexity of phage–bacterial interactions in the intestine. Further implementation of these methods should reveal insights into how phages contribute to human health. Although this work has deepened our understanding of phage–bacteria connections in the intestinal microbiota, some limitations and areas for improvement should be noted. Although this method does not rely on traditional culture and enrichment techniques to identify phage–bacterial host interactions, the technique may suffer from loss of bacterial diversity. Sample collection in this current iteration of the methodology affects viable obligate anaerobes. Additionally, the method measures phage adsorption to define an interaction, which may not be mutually exclusive to viable bacterial cells. The use of non-discriminating nucleic acid staining means that this method is broadly applicable to both single- and doublestranded DNA and RNA viruses. While the majority of intestinal phages contain doublestranded DNA genomes, the inclusion of RNA phages would expand the breadth of phage–bacterial connections in the intestine. Considering RNA phages remain among the most poorly characterized phages of the intestinal microbiota, the methodology described in this study could be used to increase the sequencing depth and annotation of these understudied viruses. Despite these limitations, this work undoubtedly advances our knowledge of phage–bacterial interactions. The

continued use of this method should shed light on how phages shape both healthy and dysbiotic microbial communities associated with human health.

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Fig. 1 |. Viral tagging reveals novel features of phage–bacteria interaction networks in the intestine.

Fluorescent dye is used to label the nucleic acid of intestinal phages, which are then mixed with complex bacterial communities to identify bacterial host interactions. This technique identified 363 novel phage–bacteria pairings in the intestine (1). Phages from unique individuals are capable of tagging bacteria from unrelated individuals (2); however, the majority of phages were discovered to be specific to a narrow spectrum of intestinal bacteria (3), suggesting that phages play a minor role in horizontal gene transfer in the intestine.