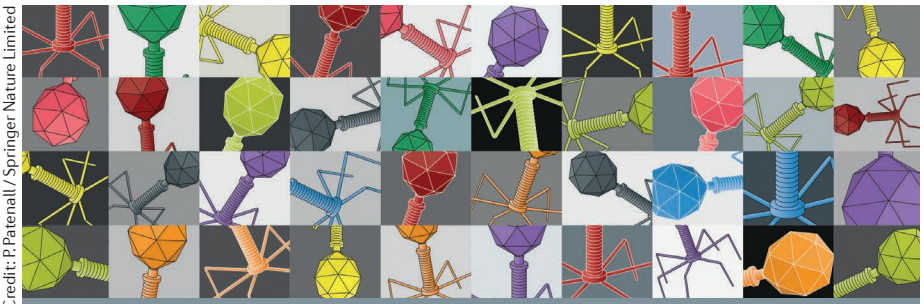

MILESTONE 8

Beyond bacteria: studies of other host-associated microorganisms

While bacteria are a major component of the human microbiota, viruses, fungi and archaea are also important members of the community, with potential effects on human health. The development of 16S ribosomal RNA gene sequencing transformed the field of microbiome research by enabling bacterial and archaea phylogenetic analyses without the need for culturing (MILESTONE 6). Fungi can also be identified and classified by sequencing a common nuclear ribosomal internal transcribed spacer region. Viruses, however, are far more challenging to isolate and sequence due to the necessity of a eukaryotic or prokaryotic host and the absence of conserved genes. The modern era of human-associated viral metagenomics actually started in the ocean. In 2001, the research group of Forest Rowher, a marine microbial ecologist at San Diego State University, published a randomized shotgun library sequencing method to analyse genomic DNA from a single bacteriophage. This was rapid, reasonably unbiased and required very little DNA input, a crucial limiting variable for sequencing. The research team further demonstrated the utility of this technique beyond single phage analysis by characterizing the more complex viral make-up of seawater and marine sediment, paving the way for analyses of human viromes and a deeper characterisation of the human microbiome.

By 2003, bacteriophages that infected individual bacterial species had been identified from human faecal waste, but the diversity and relative abundance of different phages remained unknown, as existing approaches were biased towards bacteria already known to be infected by phages. By using the linker-amplified shotgun library approach, Rowher's research group provided the first quantitative description of the composition of the uncultured virome in human faeces collected from a single healthy adult. The majority of phage sequence matches were from temperate phages, which commonly integrate into the host bacterial genome. The faecal virome was also dominated by phages known to infect Gram-positive bacteria, in keeping with prior data on faecal bacterial content.

Viral metagenomics has continued to advance, with higher-throughput techniques enabling more rapid virus discovery and classification in both healthy and diseased tissues, and helping us to understand the role of commensal and pathogenic viruses in the context of the wider microbiome.

In a study published in 2010, by Jeffrey Gordon and Rowher, faecal viromes of healthy adult female twins and their mothers were shown to be individually distinct and stable over the course of a year, in agreement with faecal bacterial data from this same cohort. Other studies have begun to elucidate the effects of disease and diet on the gut virome, finding that phage composition became increasingly similar between individuals on the same diet, and that significant expansion of one phage order is associated with Crohn's disease and ulcerative colitis. Viral microbiome signatures may therefore be associated with environmental influences as well as disease progression, including in tissues beyond the gut.

Shotgun metagenomics has facilitated a more functional and interconnected perspective of the microbiome, with the contributions of fungi and archaea also becoming increasingly understood. The human mycobiome (the fungal community of humans) has also been fruitfully studied. A number of recent studies have highlighted the crucial roles of fungi in both healthy and disease states. For example, enteric commensal bacteria and fungi may have redundant protective and tolerizing functions in the context of regulating the immune response, suggesting that gut homeostasis can be retained through the mycobiome even in the absence of 'good' bacteria. In inflammatory bowel diseases, however, mycobiome dysbiosis contributes to disease progression, with fungi enriched at the expense of bacteria. These data, along with other studies, suggest a complex interplay between fungi and bacteria.

Recently, the human archaeome (the community of human-associated archaea) has attracted interest due to a number of observations, including recognition of the human gut archaeon *Methanosphaera stadtmanae* by the immune system and the discovery of previously undetected human-associated archaea. Methanogenic archaea are amongst the most abundant microorganisms in the human gut and sometimes outnumber even the most abundant bacterial species. In 2004, methanogenic archaea were found to be associated with the onset of periodontal disease, identifying the first link between archaea and human disease. As with bacteria, viruses and fungi, archaea have been isolated from various human anatomical sites, including the gut, skin, vagina and oral cavity. Yet, owing to fundamental differences in biology, they have often remained undetected in microbiome surveys, warranting further investigation of the human archaeome and the role of archaea in human health and disease.

As identification and characterization of the human virome, mycobiome and archaeome in various tissues and disease states continues to improve, it will be increasingly important to delineate function and how these other 'omes' interact as a community to preserve or dysregulate human health.

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