The human gut virome: form and function

Lesley A Ogilvie[#] and Brian V Jones[#]

School of Pharmacy and Biomolecular Sciences, University of Brighton, UK, BN2 4GJ

#Correspondence should be addressed to either:

Lesley A Ogilvie: ogilvie.lesley@gmail.com

Brian V Jones: b.v.jones@brighton.ac.uk

Running title: Phage form and function

Key words: bacteriophage; human gut virome; human gut phageome

Abstract

Advances in next generation sequencing technologies and the application of metagenomic approaches have fuelled an exponential increase in our understanding of the human gut microbiome. These approaches are now also illuminating features of the diverse and abundant collection of viruses (termed the virome) subsisting with the microbial ecosystems residing within the human holobiont. Here we focus on the current and emerging knowledge of the human gut virome, with a particular focus on viruses infecting bacteria (bacteriophage or phage), which are a dominant component of this viral community. We summarise current insights regarding the form and function of this 'human gut phageome' and highlight promising avenues for future research. In doing so we discuss the potential for phage to drive ecological functioning and evolutionary change within this important microbial ecosystem, their contribution to modulation of host-microbiome interactions and stability of the community as a whole, as well as the potential role of the phageome in human health and disease. We also consider the emerging concepts of a 'core healthy gut phageome' and the putative existence of 'viral enterotypes' and 'viral dysbiosis'.

Introduction

In the last decade we have seen an exponential increase in our understanding of the human microbiome, which may be defined as the collection of microbes associated with the human body and their genetic content. The acceleration in our understanding of this ecosystem has been largely fuelled by advances in next generation sequencing technologies, and the application of metagenomic approaches. These new tools allow detailed, culture-independent, interrogation of the human-microbial landscape at an unprecedented scale, enabling the biological significance and diversity of these human associated microbial communities to be uncovered (1–8).

One of the most well-characterised and densely populated areas of the human microbiome is the adult human gastrointestinal tract. Approximately 10^{13} bacterial cells (9) and an average of ~160 distinct species may reside in the adult human alimentary tract (predominantly in the colon), with over 1000 different bacterial species in total associated with the human gut microbiome (5). The gut microbial community is now accepted to be intimately involved in our health and well-being, providing a range of beneficial functions such as extraction of additional energy from our diet, shaping the development of our immune systems, providing protection from invading pathogens, and has emerging roles in modulating mood, behaviour, neurocognitive development, and even the ageing process (1–4,8,10–15). Imbalances in the make-up of the gut microbiome - also termed dysbiosis – is now increasingly linked with a wide spectrum of diseases and disorders (both gut associated and those relating to extra-intestinal organ systems). These range from Inflammatory bowel diseases and cancer, to metabolic disorders, obesity, and even autism and Alzheimers (16,17). Emphasis is

now being placed on delineating whether dysbiosis of the microbiome is a cause or consequence of some of these diseases, and how manipulation of the gut microbiome may aid prophylaxis, diagnosis, or treatment (18,19).

However, as with microbial ecosystems extant in other habitats, the gut microbiome is itself host to another less well studied and explored community of non-cellular microbes. Metagenomic sequencing efforts are also now revealing the range of viruses associated with the human gut microbiome, termed the human gut virome (20,21). This viral community encompasses an abundant and diverse collection of viruses that infect every domain of life (Eukaryota, Archaea, and Bacteria), but perhaps not surprisingly, is dominated by viruses that infect and replicate within bacterial cells (bacteriophage or phage) (20,21). Because of this dominance of phage, the term “phageome” is often used to refer specifically to the bacteriophage fraction of the gut virome.

Although the study of the gut virome or phageome is far less advanced than that of the underlying microbiome, a range of important roles and functions have already been attributed to phage in non-host associated bacterial ecosystems (e.g. marine or freshwater environments), which are likely to also apply in some shape or form to the gut virome. These include the transfer of genes between different bacterial strains or species, modulation of community structure and corresponding functional outputs, and provision of accessory functions that directly benefit bacterial hosts (22–24). In the context of host-associated microbial ecosystems, the capacity of phage to endow bacterial hosts with new abilities is of additional significance when traits that may directly impact host health are considered, including toxin synthesis, production of

virulence factors and antibiotic resistance genes (20,21,25–32). More generally, the ability to infect and kill their bacterial hosts also give phage the potential to modulate bacterial community structure and destabilise the gut microbiome, which may in turn diminish or obviate benefits provided by the gut microbiome, or lead to deleterious host-microbe interactions (23,33,34). In contrast, recent research is also revealing how phage within the gut virome may play important fundamental functions in microbiome maintenance and recovery from antibiotic perturbation, and the potential for these viruses to enter direct symbiotic relationships with the higher human host (31,35,36).

As the role of the virome in the development and functioning of the gut microbial community is starting to be uncovered, evidence is accumulating that this viral community also reflects the co-evolution of host and microbe within the gut, driving diversity and functionality, and that specific phage may be unique to or enriched within the gut ecosystem (23,34,37–40). Recognition of these attributes and the potential of phage to drive ecological functioning and evolutionary change (23,34,37,39,40), has understandably ignited interest in investigating the role of these prokaryotic viruses within the human gut virome and as part of the human gut microbiome as a whole. It is also fitting that the concept of dysbiosis, and the impacts of such perturbations on human health, have begun to be considered from the perspective of the virome or phageome, and there is a growing consensus that this concept should also be extended to the phage component of the gut ecosystem (see reviews by (41–43). Here we review current knowledge of the human gut virome (with a particular focus on the phageome), and summarise new insights into its form and function.

Virome Structure and ecological dynamics

In recent years, studies of the human gut virome have mainly focused on the analysis of virus like particles (VLPs) purified from faecal samples, and the application of high throughput metagenomic approaches to characterise these (20,21,44,45). These studies have provided much insight into the diversity and structure of the gut virome, which is likely to reflect the underlying diversity of the bacterial microbiome (5).

Our current knowledge indicates the bacteriophage component of the gut virome (or the phageome) to be dominated by double stranded DNA phages of the order Caudovirales (Podoviridae, Siphoviridae, and Myoviridae) as well as the single stranded DNA containing members of the family *Microviridae* (20,21). These key virotypes mostly infect bacteria belonging to the most prevalent phyla within the gut, comprising members of the Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria (42). RNA viruses have also been identified, but these appear to represent only a minor fraction of the viral community based on available studies, and are thought to be mainly allocthonous plant infecting viruses ingested with food, rather than perhaps true constituents of the gut virome (27). The adult gut virome may be dominated by just one or a few different virotypes (20,21) and is characterised by a high degree of stability in terms of its structure over time, with temporal tracking of gut virotypes revealing the retention of between 80 to 95% of virotypes over a period of one to 2.5 years (20,21).

In terms of diversity, available estimates suggest a healthy human gut is populated by between 35-2800 actively replicating viruses (46), but that phage genome diversity is lower in the gut compared to environments such as the ocean or hot springs and even within other host associated sites, such the lung and oral cavity (47).

A large proportion of sequences identified in metagenomic surveys, however, are without close homologues within public databases, reflecting the largely uncharacterised nature of the phage gene-space in most microbial habitats, including the mammalian gut. In terms of the gut phageome specifically, the novelty inherent in this viral community was clearly highlighted in the landmark study published by Reyes and co-workers, where approximately 80% of reads lacked notable homology to known viruses in public repositories (20).

In contrast to other environments, in which phage are known to outnumber their bacterial hosts by an order of magnitude (22), phage are thought to exist at more equitable ratios within the human gut (20), with an estimated 10^{9}-10^{12} VLPs (48,49) in comparison to an estimated 10^{11} bacteria per gram of faecal material (9,50). However, phage can accumulate to higher densities at mucosal surfaces, significantly outnumbering their hosts in these niches (approximately 20:1 in the murine intestine) (35). Recent work is also revealing that, as with characterisation of the microbiome in general, extraction protocols may influence the estimation of VLP numbers as well as delineation of community structure derived from subsequent high throughput sequencing efforts (48,49). This is also highlighted by studies that have shown significant fractions of the gut phageome may be accessed *via* the analyses of conventional metagenomic datasets, which are based on the extraction and sequencing of bacterial DNA (21,32,51,52). Studies of the gut phageome through these alternative approaches to VLP analysis, have also suggested that standard metagenomes may provide access to particular groups of phage not well represented by VLP-based libraries, which will presumably be dominated by actively replicating phage (32,41).

Metagenomic analyses have also provided insight into the lifestyles of human gut phage, and provided evidence that the human gut phageome is largely composed of temperate phage, as indicated by the frequency of integrase genes in human gut viral metagenomes (20,21). Temperate phage undergo a lysogenic cycle of reproduction, where phage genetic material is integrated into the genome of bacterial hosts cells to form a dormant prophage, or persists as a dormant episomal element, and replicated with the host genome during bacterial reproduction. The potential dominance of temperate phage in the gut has led to hypotheses that the “piggyback-the-winner” model of phage ecological dynamics pervades in the human gut virome (53,54), in which lysogenic replication is predicted to dominate under conditions of high nutrient availability and bacterial growth. This is in stark contrast to non-host associated environments, in which virulent phage and lytic reproduction appear to dominate, leading to ‘kill the winner’ (or Lotka-Volterra) phage-host dynamics, that manifest as lagged boom-bust cycles of phage-host abundance (55,56).

When considered from an evolutionary perspective, the proposed dominance of temperate phage in the gut community is congruent with the reported stability of the gut microbiome in adults (20,21,57,58), and the top-down selective pressure for a functionally stable gut microbiome that is hypothesised to be exerted by the higher mammalian host (4). However, a dominance of temperate, lysogenic phage is also in line with studies that have shown access to novel fractions of the phageome through interrogation of metagenomic datasets derived from bacterial DNA extracts (32,51,52).

Other models of phage ecological dynamics may also be of relevance to the gut virome, which include “fluctuating-selection-dynamics” (FSD) and “arms-race-dynamics” (ARD) (39,59–61). In the FSD model bacteria and phage populations continually fluctuate though lagged cycles of expansion and contraction, but without total elimination of phage hosts, and instead bacterial diversity is maintained and stable phage communities are established, which is also congruent with features of the gut virome (39,59–61). In contrast, ARD are evident in the co-evolution of lytic phage with host bacteria, in which development of host defence systems to avoid predation by phage are continually countered by reciprocal developments of new infection strategies in phage, leading to what has been termed an evolutionary arms race (39,59). Given the apparent dominance of temperate phage in the human gut virome, ARD seems less likely to be a major ecological dynamic in the human gut habitat, but may be relevant to a limited number of specific host-phage systems, or during situations where bacterial diversity is reduced and underlying ecology of the microbiome altered with respect to the stable adult community. This may include situations such as dysbiosis during disease, and conceivably the infant or elderly gut communities. Readers are directed to excellent reviews (39,59) for more in-depth analysis of phage-bacterial dynamics and effects on the diversity and structure of this ecosystem.

Inter-personal variation and potential for ‘viral enterotypes’

As with the microbiome, a considerable inter-individual variability in phage diversity is evident in human gut viromes studied to date (20,21,32). This variability is exemplified by the analysis conducted by Reyes et al, who evaluted the representation of highly abundant phage genomes (partial and complete) assembled from metagenomic reads

(88 in total), revealing that only 8 were found in more than one individual (20). This high person-to-person variability most likely stems from the ability of phage to undergo rapid evolution to form new virotypes (45), and the associated inter-personal variability of host bacterial species within the gut microbiome (5). In contrast, studies focusing on the microbiome in early infancy have indicted the virome to be low in diversity but highly dynamic during the very early developmental stages (26). Using epifluorescence microscopy, viral particles were found to be absent in the first stool samples from infants, but rapidly appear in the gut and reached levels of up to 10^8 particles per gram of faeces by the end of the first week of life (26). A shift in microbiome structure and reduction in species diversity is also now well documented in elderly individuals, but there is currently a paucity of information regarding the gut virome structure in old age, and whether constituent phage may be involved in changes to the gut community seen with ageing.

Despite the inter-individual differences in adult gut microbiomes and phageomes, the structure of the gut phageome can converge due to diet (21) and individuals (related or unrelated) who live in the same household will share a certain proportion of their gut virome, raising the potential for viral transmission between individuals in close contact (62). More recently, however, a conservation of virotypes in multiple individuals of diverse geographical origin (32,51,52,63) has been detected leading to the hypothesis of a core phageome in healthy individuals (42,63). Despite the reported high level of individuality between human gut viromes (20,21), a set of 23 ‘core’ phages have been identified within more than half of (geographically dispersed) individuals surveyed (n= 62) (63).

Such shared patterns of phage distribution have opened up the possibility of the existence of viral “enterotypes” (32), akin to observations from the bacterial component of the human gut microbiome, in which multiple individuals could be stratified according to their gut microbial composition into several “enterotypes” (7). As further studies and data accumulate, the nature of, or indeed the existence of these putative enterotypes will be resolved (32,64). Nevertheless, consideration of the human gut phageome should be an integral part of investigations that focus on defining structural or functional aspects of the gut microbiome, and their relevance to human health (32,64).

Functional capacity within the phageome and potential for modulation of host-microbiome interactions

The idea that phage can also play a role in the health status of individuals by influencing microbiome structure or the phenotypes of host bacterial species, has also started to emerge (41,42,65,47,66). Significant shifts in community structure, including the balance of lytic versus lysogenic phage (see (42), for insightful discussion of this topic), have been observed in conditions such as inflammatory bowel diseases, autoimmunity, leukaemia and diabetes (67–71). How phage may contribute to modifications of underlying bacterial communities, and shifts from equilibrium with the human host to sub-optimal, unhealthy, or pathogenic interactions, is a subject of intense investigation and is comprehensively reviewed by De Paepe and colleagues (47).

In one scenario, bacterial dysbiosis may result from environmental stressors triggering prophage induction more often in mutualistic bacteria than opportunistic pathogens

shifting the ratio of these bacterial types, termed the ‘community shuffling hypothesis’ (66). Phage within the gut also have the potential to modulate the immune response, either indirectly by modifying bacterial antigens or directly if phage particles are phagocytosed or infiltrate the intracellular environment (47,71,72). Additionally, a potentially unique role in gut health has also been highlighted for phage by recent work in mice revealing that the bacterial and virome components of the murine gut exhibited distinct responses to dietary intervention (73), results that the authors note are reminiscent of responses noted in Crohn’s disease patients (69).

There is also considerable potential for phage to augment the functional repertoire of species comprising the microbiome, and confer particular advantages and new capabilities to host species or strains. This in turn could influence the interaction of these species with the human host, or their competitive fitness within the gut microbiome. Analysis of data generated from human-gut derived VLP libraries, as well as phage-orientated dissection of whole community gut metagenomes, has identified a broad functional potential ranging from bacteriocins, lysins, holins, restriction modification systems, virulence factors, genes associated with energy transfer and key biosynthetic pathways in bacterial hosts, as well as antibiotic resistance genes (20,21,25,26,31,32,74). A functional repertoire that places phage alongside other mobile genetic elements such as plasmids, with regard to the potential to introduce new traits to their bacterial hosts and develop new functional capacity. The ability of temperate phage to form long-term genetic symbioses with their host bacteria, inserted as prophage within host chromosomes, is likely to be an important source of genetic variation and diversity within the human gut, driving genetic exchange and altering phenotypes *via* lysogenic conversion. The apparent dominance of the temperate

lifestyle within the gut therefore has profound implications for community development and evolution (as outlined in Table 1), as well as functional capacity of gut microbes (23,75,76).

There are numerous examples from studies of pathogenic gut bacteria, of phage facilitating the transfer of genetic material and/or encoding functions that may be beneficial for their host bacteria. This includes the wide range of phage-encoded virulence factors and toxins encoded by prophage (77) that lead to major infectious diseases. A particularly pertinent example to the gut ecosystem is the emergence of shiga toxin producing enteropathogenic *E. coli* strains that can cause severe disease, in which toxin production has been acquired through infection with lysogenic phage (78).

A number of studies are now also revealing a diverse and significant phage-encoded antibiotic resistance gene pool within the human gut (20,21,79), which has been shown to be viable, mobile (31) and widely distributed within multiple individuals of diverse geographical origin (32). Moreover the importance of phage and the mechanisms used for acquiring and distributing antibiotic resistance genes has also been highlighted (80,81). Nevertheless, the true extent of the phage-encoded antibiotic resistance pool needs to be charted in a systematic and stringent manner.

In this context, Enault and colleagues (82) have suggested that the use of non-conservative thresholds within many bioinformatic-based surveys may have led to vast overestimates of phage-encoded antibiotic resistance genes in virome studies. The authors argue that the main route of transfer will be via generalised transduction, which

relies on errors in the packaging of non-phage DNA, intrinsically limiting the role of phage in dissemination of these genes to movement between species or strains infected by the transducing phage (82). How these observations can be reconciled with the reported expansion of the phage resistome following antibiotic perturbation ((31); see also *Community-level functions of the gut phageome*), has yet to be determined. However, it is clear that further investigations of the mechanisms of phage-encoded gene transfer of clinically important traits such as antibiotic resistance are required to provide a more cohesive view of gene transfer and phage-bacteria interactions within the gut. Such studies will be inevitably aided by the isolation and genomic characterisation of phage originating from the human gut and in particular those infecting key members of this microbiome.

Aside from the acquisition of accessory functions, such as virulence or antibiotic resistance genes, phage also have the potential to influence strain-strain competition within the gut ecosystem by facilitating the elimination of strains that would otherwise compete with bacterial hosts for resources. This derives from situations where prophage confer immunity to host strains from further infections by the same or closely related phage (30; Table 1). This contribution of phage to strain-strain competition could also play a role in facilitating maintenance of particular strains within the gut microbiome, or potentially in the ability of less desirable strains to displace beneficial members of the gut community. An elegant example of phage-mediated inter-strain warfare has recently been described in *Enterococcus faecalis* V583 (30). Prophage carried by this human gut colonising strain have been found to be crucial in allowing it to persist in the intestine, and avoid competitive exclusion by other strains. In this case, the prophage carried by V583 produces a constitutive low-level shedding of viral

particles from the host cell (also referred to as a chronic replication cycle). The phage particles release by V583 can infect and kill competing strains, but V583 host cells are rendered immune by virtue of their prophage lysogen (30). Alternatively, carriage of cryptic phages – a prophage that has lost the ability to enter the lytic cycle or produce phage particles – can be beneficial for surviving adverse conditions. The carriage of cryptic phage by *E.coli* enables the host bacterium to withstand multiple stressors such as oxidative, pH and antibiotic stressors and influences growth and biofilm formation (83; Table 1).

Community-level functions of the gut phageome

There is also considerable interest in the potential for the human gut phageome to play important and fundamental roles in the maintenance of a stable gut microbiome, and the scope for these viruses to provide direct benefits to the human host, outside of those that may be gained indirectly through effects on the bacterial gut community (as summarised in Table 1; see (84) for discussion of the beneficial impacts of viruses).

The potential for phage to contribute to adaptability and recovery of the gut microbiome has been recently highlighted by Modi and co-workers, who detail the functional resilience of the community following antibiotic exposure (31). Gut-derived phage metagenomes from mice subjected to stress in the form of antibiotic treatment, were enriched in genes encoding functions involved in host metabolism, such as metabolism of cofactors and vitamins, as well as carbohydrate degrading enzymes and antibiotic resistance determinants. The authors suggested that this phage-encoded accessory gene pool could act as a community-based mechanism to preserve the functional robustness of the gut microbiome during perturbation, acting

as a buffer of functions that are essential for the host-microbe relationship and that may become depleted during insults such as antibiotic treatment. The perturbation also led to a reported expansion of interactions between phage and bacteria that would likely increase the possibility of gene exchange, and further affirm the well-known role of phage in horizontal gene transfer. See Sun & Relman (85), for further analysis of this topic.

Moving beyond the direct phage-bacteria co-evolutionary relationship, the potential for phage to directly enter into a symbiotic relationship with higher metazoan hosts has also been documented in some host-microbe relationships, and theorised to occur within the human gut (35,36; Table 1). In these phage-metazoan relationships, protecting or enhancing the fitness of the metazoan benefits the phage by maintaining the habitat, and therefore population, of host bacterial species in which it replicates. A good example of this co-evolutionary triangle has been identified through studies of the aphid symbiont *Hamiltonia defensa* (36), which confers protection to aphid hosts from attack by parasitoid wasps. Detailed analyses of this symbiosis has revealed the toxins responsible for the *H. defensa* protective effect are encoded by lysogenic phage that infect this species, and strains of *H. defensa* lacking this phage lysogen provide no protective effect (36).

In the context of the human gut microbiome and associated virome, hypotheses for how this collection of phage may conceivably provide direct benefits to the human host are also emerging. The apparent enrichment of phage in the mucus layer covering intestinal epithelial cells in the mammalian gut, has led Barr and co-workers (35) to propose that phage accumulating in this region may provide important benefits for the

host by controlling the bacterial population in the mucus layer, and potentially providing protection from pathogens. Within this theory, phage embedded within the intestinal mucus layer are essentially hypothesised to provide a form of “non-host-derived immunity”, and enter into a direct symbiosis with the higher mammalian host (35). If true, this function of the gut virome opens further routes though which gut-associated phage may not only influence aspects of human well-being, but also potentially aspects of development, and would suggest that understanding the capacity or potential for “viral dysbiosis” should be given the same emphasis as perturbation of the bacterial component of this community. An exhaustive review of the concepts and potential consequences of host-microbiome-virome co-evolution is beyond the scope of this review but we refer the reader to excellent reviews by (39,59).

Applications

Since their initial discovery in the early twentieth century independently by Frederick Twort in 1915 (86) and Felix d’Herelle in 1917 (87), phage have been viewed in terms of human application, in the first instance for their antimicrobial properties. There is a long history of lytic phage and their encoded products being used to kill pathogenic bacterial species, an approach currently entering a renaissance in this critical era of antibiotic resistance. See (88–91) for excellent discussions on the topic. In the context of biotechnological and biomedical applications, phage also have a long history as work horses of molecular biology research, as well as delivery vehicles for vaccines and gene therapy (92). As our ability to chart and characterise the human gut phageome (and virome as a whole) continues, however, new biomedical and biotechnological avenues of application are being pursued.

Recognition that dysbiosis may also incorporate the phage component of the human gut ecosystem also opens up novel options for using phage as prognostic or predictive markers of disease (41,43). The notion that individuals could be stratified according to their virome structure (32) creates potential to develop diagnostics, prognostics and treatment approaches (64), expanding the microbiome for personalised medicine applications (7,93). In tandem, the idea that phage have the potential to contribute to recovery and resilience of a gut community following perturbation (23,31) forms the foundation of using virome components for targeted interventions. In this context there has been much interested in the genetic engineering of phage for microbiome restructuring and selective elimination of bacterial strains (94–96).

The specificity of phage for the human gut (38,97) can also be leveraged for the development of microbial source tracking tools (MST), which permit detection of faecal pollution in surface and ground waters. Faecal contamination poses a significant risk to human health and therefore there has been much interest in developing rapid and sensitive culture-independent methods that can detect human faecal indicator phage within environmental samples. Phage persist longer in the environment than host bacteria and often at higher numbers making them a potentially more sensitive source tracking tool (98–101). In tandem with improvements in next generation sequencing technologies, there is also potential to develop metagenomic approaches to MST, e.g. (102,103).The combination of MST with the high resolution provided by metagenomics.-based analysis of whole microbial communities potentially enabling identification of habitat associated genetic patterns – or an ecogenomic signature - that could be used to differentiate ecosystems.

Conclusions

Knowledge around the human gut virome is now accumulating at an increasingly rapid pace. In particular next generation sequencing technologies have given us the ability to access the human gut phageome (and virome in general), to gain fundamental insight into the form and function of this viral assemblage. Studies to date are revealing a diverse and abundant viral ecosystem that is being increasingly recognised as an important facet of the gut microbiome. As progress continues in our ability to characterise the human gut virome so does our ability to harness its power for biomedical and biotechnological application. Further understanding of the host-phage dynamic is crucial in moving the field forward, with more emphasis on temporal and multi-level studies that take a more inclusive view of the human gut virome, alongside the other components of the human gut microbiome. Integral to these efforts, and the more meaningful interpretation of data derived from metagenomic approaches to study the gut virome, will be continued efforts to pursue more traditional approaches to isolate, propagate, and characterise phage comprising the human gut phageome.

Summary Points

- Advances in next generation sequencing technologies are allowing us to illuminate the human gut virome, the diverse and abundant collection of viruses associated with the human gut microbiome.

- We focus here on the form and function of the viruses infecting bacteria (bacteriophage or phage), which are a dominant component of the human gut virome.

- Key points of discussion include the potential role of the ‘phageome’ in driving ecological functioning and evolutionary change with the human gut microbiome and how they could contribute to modulation of host-microbiome interactions and stability of the community as a whole, as well as their potential role in health and disease.

- Emerging concepts of a ‘core healthy gut phageome’, the putative existence of ‘viral enterotypes’ and ‘viral dysbiosis’ are considered.

Box 1

Glossary	
Bacteriophage	Viruses which infect and complete their life cycle within bacterial cells
Human gut virome	The collection of viruses associated with the human gut microbiome
Phageome	The genetic composition of the bacteriophage fraction of a microbial community
Prophage	Term to describe a phage genome which is integrated into a bacterial chromosome. Can also exist externally to the bacterial chromosome as an episomal element. The prophage is a non-infective precursor phage.
Temperate	When phage have the ability to enter a lysogenic cycle of replication.
Lysogeny	Ability of phage to integrate into host bacterium DNA to become a prophage. The prophage is copied each time the bacterial cell divides.
Lysogenic conversion	Expression of prophage genes within the host genome affecting the phenotype of the host cell.
Composite phage	A phage derived from two distinct chromosomally encoded prophage elements

References

1. Rakoff-Nahoum S, Foster KR, Comstock LE. The evolution of cooperation within the gut microbiota. Nature 2016;533(7602):255–9.
2. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of Commensal Microflora by Toll-Like Receptors Is Required for Intestinal Homeostasis. Cell 2004;118(2):229–41.
3. Bäckhed F, Ley RE, Sonnenburg JL, Peterson D a, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005 ;307(5717):1915–20.
4. Ley RE, Peterson D a, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006 Feb;124(4):837–48.
5. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing: Commentary. Nature 2010 464(7285):28.
6. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux J-J, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci 2008 2017;105(43):16731–6.
7. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature 2011;473(7346):174–80.
8. Fransen F, van Beek AA, Borghuis T, Meijer B, Hugenholtz F, van der Gaast-de Jongh C, et al. The Impact of Gut Microbiota on Gender-Specific Differences in Immunity. Front Immunol. 2017;8:754.
9. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016;14(8):e1002533.
10. Zoetendal EG, Vaughan EE, de Vos WM. A microbial world within us. Mol

Microbiol. 2006;59(6):1639–50.

11. Dinan TG, Stilling RM, Stanton C, Cryan JF. Collective unconscious: How gut microbes shape human behavior. J Psychiatr Res. 2015;63:1–9.

12. Han B, Sivaramakrishnan P, Lin C-CJ, Neve IAA, He J, Tay LWR, et al. Microbial Genetic Composition Tunes Host Longevity. Cell 2017;169(7):1249–1262.e13.

13. Heintz C, Mair W. You are what you host: microbiome modulation of the aging process. Cell 2014; 156(3):408–11.

14. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013;155(7):1451–63.

15. Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci 2011;108(7):3047–52.

16. Dinan TG, Cryan JF. The Microbiome-Gut-Brain Axis in Health and Disease. Gastroenterol Clin North Am 2017;46(1):77–89.

17. Lynch S V., Pedersen O. The Human Intestinal Microbiome in Health and Disease.N Engl J Med 2016;375(24):2369–79.

18. Amitay EL, Werner S, Vital M, Pieper DH, Höfler D, Gierse I-J, et al. Fusobacterium and colorectal cancer: causal factor or passenger? Results from a large colorectal cancer screening study. Carcinogenesis 2017; 38(8):781-788.

19. Sommer F, Anderson JM, Bharti R, Raes J, Rosenstiel P. The resilience of the intestinal microbiota influences health and disease. Nat Rev Microbiol. 2017; 15(10):630-638.

20. Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature. 2010 ;466(7304):334–8.
21. Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, et al. The human gut virome: Inter-individual variation and dynamic response to diet. Genome Res. 2011;21(10):1616–25.
22. Suttle CA. Viruses in the sea. Nature. 2005 Sep;437(7057):356–61.
23. Brüssow H, Canchaya C, Hardt W-D. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol Mol Biol Rev. 2004;68(3):560–602.
24. Wommack KE, Colwell RR. Virioplankton: viruses in aquatic ecosystems. Microbiol Mol Biol Rev. 2000;64(1):69–114.
25. Breitbart M, Hewson I, Felts B, Mahaffy JM, Nulton J, Salamon P, et al. Metagenomic analyses of an uncultured viral community from human feces. J Bacteriol. 2003;185(20):6220–3.
26. Breitbart M, Haynes M, Kelley S, Angly F, Edwards R a, Felts B, et al. Viral diversity and dynamics in an infant gut. Res Microbiol. 2008;159(5):367–73.
27. Zhang T, Breitbart M, Lee WH, Run J-Q, Wei CL, Soh SWL, et al. RNA viral community in human feces: prevalence of plant pathogenic viruses. Dangl J, editor. PLoS Biol. 2006;4(1):e3.
28. Colomer-Lluch M, Imamovic L, Jofre J, Muniesa M. Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs, and poultry. Antimicrob Agents Chemother. 2011;55(10):4908–11.
29. Kim K-H, Chang H-W, Nam Y-D, Roh SW, Kim M-S, Sung Y, et al. Amplification of uncultured single-stranded DNA viruses from rice paddy soil.

Appl Environ Microbiol. 2008;74(19):5975–85.

30. Duerkop BA, Clements C V, Rollins D, Rodrigues JLM, Hooper L V. A composite bacteriophage alters colonization by an intestinal commensal bacterium. Proc Natl Acad Sci U S A. 2012;109(43):17621–6.

31. Modi SR, Lee HH, Spina CS, Collins JJ. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Nature 2013;499(7457):219–22.

32. Ogilvie LA, Bowler LD, Caplin J, Dedi C, Diston D, Cheek E, et al. Genome signature-based dissection of human gut metagenomes to extract subliminal viral sequences. Nat Commun. 2013;4:2420.

33. Lepage P, Leclerc MC, Joossens M, Mondot S, Blottière HM, Raes J, et al. A metagenomic insight into our gut's microbiome. Gut 2013;62(1):146–58.

34. Koskella B, Brockhurst MA. Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. FEMS Microbiol Rev. 2014;38(5):916–31.

35. Barr JJ, Auro R, Furlan M, Whiteson KL, Erb ML, Pogliano J, et al. Bacteriophage adhering to mucus provide a non-host-derived immunity. Proc Natl Acad Sci. 2013;110(26):10771–6.

36. Oliver KM, Degnan PH, Hunter MS, Moran NA. Bacteriophages Encode Factors Required for Protection in a Symbiotic Mutualism. Science 2009;325(5943):992–4.

37. Koskella B, Lively CM. Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. Evolution 2009;63(9):2213–21.

38. Ogilvie LA, Caplin J, Dedi C, Diston D, Cheek E, Bowler L, et al. Comparative

(meta)genomic analysis and ecological profiling of human gut-specific bacteriophage φB124-14. PLoS One 2012;7(4):e35053.

39. Scanlan PD. Bacteria–Bacteriophage Coevolution in the Human Gut: Implications for Microbial Diversity and Functionality. Trends Microbiol. 2017;25(8):614–23.

40. Ogilvie LA, Firouzmand S, Jones B V. Evolutionary, ecological and biotechnological perspectives on plasmids resident in the human gut mobile metagenome. Bioeng Bugs. 2012;3(1):13–31.

41. Ogilvie LA, Jones B V. The human gut virome: a multifaceted majority. Front Microbiol. 2015;6:918.

42. Manrique P, Dills M, Young M. The Human Gut Phage Community and Its Implications for Health and Disease. Viruses 2017;9(6):141.

43. Dalmasso M, Hill C, Ross RP. Exploiting gut bacteriophages for human health. Trends Microbiol. 2014;22(7):399–405.

44. Reyes A, Semenkovich NP, Whiteson K, Rohwer F, Gordon JI. Going viral: next-generation sequencing applied to phage populations in the human gut. Nat Rev Microbiol. 2012;10(9):607–17.

45. Minot S. Rapid evolution of the human gut virome. Proc Natl Acad Sci USA. 2013;110(30):12450-5.

46. Manrique P, Bolduc B, Walk ST, van der Oost J, de Vos WM, Young MJ. Healthy human gut phageome. Proc Natl Acad Sci. 2016;113(37):10400–5.

47. De Paepe M, Leclerc M, Tinsley CR, Petit M-A. Bacteriophages: an underestimated role in human and animal health? Front Cell Infect Microbiol. 2014;4:39.

48. Hoyles L, McCartney AL, Neve H, Gibson GR, Sanderson JD, Heller KJ, et al.

595 Characterization of virus-like particles associated with the human faecal and
596 caecal microbiota. Res Microbiol. 2014;165(10):803–12.
597 49. Castro-Mejía JL, Muhammed MK, Kot W, Neve H, Franz CMAP, Hansen LH,
598 et al. Optimizing protocols for extraction of bacteriophages prior to
599 metagenomic analyses of phage communities in the human gut. Microbiome.
600 2015;3(1):64.
601 50. Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting
602 the Ratio of Bacterial to Host Cells in Humans. Cell. 2016;164(3):337–40.
603 51. Stern A, Mick E, Tirosh I, Sagy O, Sorek R. CRISPR targeting reveals a
604 reservoir of common phages associated with the human gut microbiome.
605 Genome Res. 2012;22(10):1985–94.
606 52. Dutilh BE, Cassman N, McNair K, Sanchez SE, Silva GGZ, Boling L, et al. A
607 highly abundant bacteriophage discovered in the unknown sequences of
608 human faecal metagenomes. Nat Commun. 2014;5:4498.
609 53. Knowles B, Silveira CB, Bailey BA, Barott K, Cantu VA, Cobián-Güemes AG,
610 et al. Lytic to temperate switching of viral communities. Nature
611 2016;531(7595):466–70.
612 54. Silveira CB, Rohwer FL. Piggyback-the-Winner in host-associated microbial
613 communities. NPJ Biofilms Microbiomes 2016; 2:16010.
614 55. Brüssow H, Hendrix RW. Phage genomics: small is beautiful. Cell
615 2002;108(1):13–6.
616 56. Thingstad TF, Bratbak G, Heldal M. Aquatic phage ecology. In: Abedon ST,
617 editor. Bacteriophage Ecology. Cambridge: Cambridge University Press p.
618 251–80.
619 57. Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman

620 AL, et al. The Long-Term Stability of the Human Gut Microbiota. Science
621 2013;341(6141):1237439–1237439.
622 58. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity,
623 stability and resilience of the human gut microbiota. Nature
624 2012;489(7415):220–30.
625 59. Mirzaei MK, Maurice CF. Ménage à trois in the human gut: interactions
626 between host, bacteria and phages. Nat Rev Microbiol. 2017;15(7):397–408.
627 60. Hall AR, Scanlan PD, Morgan AD, Buckling A. Host-parasite coevolutionary
628 arms races give way to fluctuating selection. Ecol Lett. 2011;14(7):635–42.
629 61. Gómez P, Buckling A. Bacteria-phage antagonistic coevolution in soil.
630 Science. 2011;332(6025):106–9.
631 62. Ly M, Jones MB, Abeles SR, Santiago-Rodriguez TM, Gao J, Chan IC, et al.
632 Transmission of viruses via our microbiomes. Microbiome 2016;4(1):64.
633 63. Manrique P, Bolduc B, Walk ST, van der Oost J, de Vos WM, Young MJ.
634 Healthy human gut phageome. Proc Natl Acad Sci U S A
635 2016;113(37):10400–5.
636 64. Ogilvie LA, Jones B V. Revisiting enterotypes: a viral perspective. Future Virol.
637 2017; 12(8):391-394.
638 65. Abeles SR, Pride DT. Molecular bases and role of viruses in the human
639 microbiome. J Mol Biol. 2014;426(23):3892–906.
640 66. Mills S, Shanahan F, Stanton C, Hill C, Coffey A, Ross RP. Movers and
641 shakers: influence of bacteriophages in shaping the mammalian gut
642 microbiota. Gut Microbes 2013; 4(1):4–16.
643 67. Furuse K, Osawa S, Kawashiro J, Tanaka R, Ozawa A, Sawamura S, et al.
644 Bacteriophage distribution in human faeces: continuous survey of healthy

645 subjects and patients with internal and leukaemic diseases. J Gen Virol.
646 1983;64 (Pt 9):2039–43.
647 68. Lepage P, Colombet J, Marteau P, Sime-Ngando T, Doré J, Leclerc M.
648 Dysbiosis in inflammatory bowel disease: a role for bacteriophages? Gut.
649 2008;57(3):424–5.
650 69. Norman JM, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, et al.
651 Disease-specific alterations in the enteric virome in inflammatory bowel
652 disease. Cell 2015;160(3):447–60.
653 70. Zhao G, Vatanen T, Droit L, Park A, Kostic AD, Poon TW, et al. Intestinal
654 virome changes precede autoimmunity in type I diabetes-susceptible children.
655 Proc Natl Acad Sci U S A 2017;201706359.
656 71. Riley PA. Bacteriophages in autoimmune disease and other inflammatory
657 conditions. Med Hypotheses 2004;62(4):493–8.
658 72. Duerkop BA, Hooper L V. Resident viruses and their interactions with the
659 immune system. Nat Immunol. 2013;14(7):654–9.
660 73. Howe A, Ringus DL, Williams RJ, Choo Z-N, Greenwald SM, Owens SM, et al.
661 Divergent responses of viral and bacterial communities in the gut microbiome
662 to dietary disturbances in mice. ISME J. 2016;10(5):1217–27.
663 74. Kim M-S, Park E-J, Roh SW, Bae J-W. Diversity and abundance of single-
664 stranded DNA viruses in human feces. Appl Environ Microbiol.
665 2011;77(22):8062–70.
666 75. Hendrix RW, Smith MC, Burns RN, Ford ME, Hatfull GF. Evolutionary
667 relationships among diverse bacteriophages and prophages: all the world’s a
668 phage. Proc Natl Acad Sci U S A. 1999;96(5):2192–7.
669 76. Nadeem A, Wahl LM. Prophage as a genetic reservoir: Promoting diversity

and driving innovation in the host community. Evolution 2017;71(8):2080-2089.

77. Abedon ST, Lejeune JT. Why bacteriophage encode exotoxins and other virulence factors. Evol Bioinform Online 2007;1:97–110.

78. Croxen MA, Finlay BB. Molecular mechanisms of Escherichia coli pathogenicity. Nat Rev Microbiol. 2009;8(1):26–38.

79. Quirós P, Colomer-Lluch M, Martínez-Castillo A, Miró E, Argente M, Jofre J, et al. Antibiotic resistance genes in the bacteriophage DNA fraction of human fecal samples. Antimicrob Agents Chemother. 2014;58(1):606–9.

80. Haaber J, Leisner JJ, Cohn MT, Catalan-Moreno A, Nielsen JB, Westh H, et al. Bacterial viruses enable their host to acquire antibiotic resistance genes from neighbouring cells. Nat Commun. 2016;7:13333.

81. Colavecchio A, Cadieux B, Lo A, Goodridge LD. Bacteriophages Contribute to the Spread of Antibiotic Resistance Genes among Foodborne Pathogens of the Enterobacteriaceae Family - A Review. Front Microbiol. 2017;8:1108.

82. Enault F, Briet A, Bouteille L, Roux S, Sullivan MB, Petit M-A. Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. ISME J. 2017;11(1):237–47.

83. Wang X, Kim Y, Ma Q, Hong SH, Pokusaeva K, Sturino JM, et al. Cryptic prophages help bacteria cope with adverse environments. Nat Commun. 2010;1(9):147.

84. Roossinck MJ. Move Over, Bacteria! Viruses Make Their Mark as Mutualistic Microbial Symbionts. Schultz-Cherry S, editor. J Virol. 2015;89(13):6532–5.

85. Sun CL, Relman DA. Microbiota’s “little helpers”: bacteriophages and antibiotic-associated responses in the gut microbiome. Genome Biol. 2013;14(7):127.

695 86. Twort FW. An investigation on the nature of ultra-microscopic viruses. Lancet
696 1915;186(4814):1241–3.
697 87. D'Hérelles F. Sur un microbe invisible antagoniste des bacilles dysentériques.
698 C R Hebd Seances Acad Sci. 1917;165(11):373–5.
699 88. Abedon ST, García P, Mullany P, Aminov R. Editorial: Phage Therapy: Past,
700 Present and Future. Front Microbiol. 2017;8:981.
701 89. Górski A, Międzybrodzki R, Weber-Dąbrowska B, Fortuna W, Letkiewicz S,
702 Rogóż P, et al. Phage Therapy: Combating Infections with Potential for
703 Evolving from Merely a Treatment for Complications to Targeting Diseases.
704 Front Microbiol. 2016;7:1515.
705 90. Nobrega FL, Costa AR, Kluskens LD, Azeredo J. Revisiting phage therapy:
706 new applications for old resources. Trends Microbiol. 2015;23(4):185–91.
707 91. Torres-Barceló C, Hochberg ME. Evolutionary Rationale for Phages as
708 Complements of Antibiotics. Trends Microbiol. 2016;24(4):249–56.
709 92. Clark JR, March JB. Bacteriophages and biotechnology: vaccines, gene
710 therapy and antibacterials. Trends Biotechnol. 2006;24(5):212–8.
711 93. Knights D, Ward TL, McKinlay CE, Miller H, Gonzalez A, McDonald D, et al.
712 Rethinking "enterotypes". Cell Host Microbe 2014;16(4):433–7.
713 94. Sheth RU, Cabral V, Chen SP, Wang HH. Manipulating Bacterial Communities
714 by in situ Microbiome Engineering. Trends Genet. 2016;32(4):189–200.
715 95. Libis VK, Bernheim AG, Basier C, Jaramillo-Riveri S, Deyell M, Aghoghogbe I,
716 et al. Silencing of antibiotic resistance in E. coli with engineered phage bearing
717 small regulatory RNAs. ACS Synth Biol. 2014;3(12):1003–6.
718 96. Lu TK, Collins JJ. Engineered bacteriophage targeting gene networks as
719 adjuvants for antibiotic therapy. Proc Natl Acad Sci U S A. 2009;106(12):4629–

720 34.
721 97. Ebdon J, Muniesa M, Taylor H. The application of a recently isolated strain of
722 Bacteroides (GB-124) to identify human sources of faecal pollution in a
723 temperate river catchment. Water Res. 2007;41(16):3683–90.
724 98. Payan A, Ebdon J, Taylor H, Gantzer C, Ottoson J, Papageorgiou GT, et al.
725 Method for Isolation of Bacteroides Bacteriophage Host Strains Suitable for
726 Tracking Sources of Fecal Pollution in Water. Appl Environ Microbiol.
727 2005;71(9):5659–62.
728 99. Gómez-Doñate M, Payán A, Cortés I, Blanch AR, Lucena F, Jofre J, et al.
729 Isolation of bacteriophage host strains of Bacteroides species suitable for
730 tracking sources of animal faecal pollution in water. Environ Microbiol.
731 2011;13(6):1622–31.
732 100. Lee JE, Lim MY, Kim SY, Lee S, Lee H, Oh H-M, et al. Molecular
733 characterization of bacteriophages for microbial source tracking in Korea. Appl
734 Environ Microbiol. 2009;75(22):7107–14.
735 101. Jofre J, Blanch AR, Lucena F, Muniesa M. Bacteriophages infecting
736 Bacteroides as a marker for microbial source tracking. Water Res. 2014;55:1–
737 11.
738 102. Knights D, Kuczynski J, Charlson ES, Zaneveld J, Mozer MC, Collman RG, et
739 al. Bayesian community-wide culture-independent microbial source tracking.
740 Nat Methods. 2011;8(9):761–3.
741 103. Tan B, Ng C, Nshimyimana JP, Loh LL, Gin KY-H, Thompson JR. Next-
742 generation sequencing (NGS) for assessment of microbial water quality:
743 current progress, challenges, and future opportunities. Front Microbiol.
744 2015;6:1027.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

This work was supported by funding from the University of Brighton (Research Challenges Grant awarded to LAO and BVJ.