The Battle Within: Interactions of Bacteriophages and Bacteria in the Gastrointestinal Tract

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Abstract

The intestinal microbiota is intimately linked to human health. Decoding the mechanisms underlying its stability in healthy subjects should uncover causes of microbiota-associated diseases and pave the way for treatment. Bacteria and bacteriophages (phages) are the most abundant biological entities in the gastrointestinal tract, where their coexistence is dynamic and affixed. Phages drive and maintain bacterial diversity by perpetuating the coevolutionary interactions with their microbial prey. This review brings together recent *in silico*, *in vitro* and *in vivo* work dissecting the complexity of phage-bacteria interactions in the intestinal microbiota, including coevolution perspectives. We define the types of dynamics encountered in the gastrointestinal tract and the parameters that affect their outcome. The impact of intestinal physiology on phage-bacterial coevolution is analysed in the light of its potential contribution to the relationship between the microbiota and human health.

Introduction

Polymicrobial communities, hereafter termed microbiota, are present in most environments and shape the ecology of these environments through metabolic reactions vital to ecosystem function. Within such communities, microbes can establish interspecies networks that can coordinate energetic pathways with effects on the environment at the global scale, as illustrated by the biogeochemical cycling processes that occur in bodies of water and the soil (Singer et al., 2017). Several microbiota also establish intimate associations with multicellular organisms, such as plants or animals, sometimes causing disease but more often developing a symbiotic coexistence of mutual benefit. The human intestinal microbiota is an example of such mutualism that is currently extensively studied, being associated with vital functions, such as digestion, the immune response and the nervous system (Belkaid and Hand, 2014; Sharon et al., 2016; Sonnenburg et al., 2005). Analyses of clinical samples have revealed that several diseases and disorders are associated with alterations in the composition of the intestinal microbiota compared to controls (Frank et al., 2007; Ley et al., 2006; Qin et al., 2012; Zhu et al., 2017). This active field of research is currently focusing on delineating the role of individual components of the microbiota, with the goal to re-establish health (Gentile and Weir, 2018).

The intestinal microbiota consist of bacteria, archaea, fungi, protists and viruses. The advent of high-throughput sequencing has opened to the door to revealing the very large microbial diversity associated to the intestinal ecosystem, which is further expanding as new cohorts are analysed worldwide (Pasolli et al., 2019). However, not all the genomic information can be assigned to a defined organism, and this is particularly true for the identification of viruses, at least in part because of the lack of common genomic markers, such as the 16S or 18S ribosomal RNA genes (Paez-Espino et al., 2016). Parasitic interactions occur within the gastrointestinal tract (GIT), as exemplified by the most abundant microbes in this environment: bacteria and the viruses that predate on them, bacteriophages (phages). The perpetuating antagonistic coevolution between the predator (phages) and the prey (bacteria) populations results in fluctuations of both these populations (Faruque et al., 2005; Koskella and Brockhurst, 2014).

The presence of viruses, including phages in particular, in the human GIT, has been known for a century, but their role in the intestinal microbiota has been little studied (d'Herelle, 1917; Reyes et al., 2012). The number of active phage species in a healthy subject has been

estimated between 35 and 2800, with more than 50% being predicted to be unique to each individual (Manrique et al., 2016; Minot et al., 2011; Reyes et al., 2010). The most abundant viral families include Myoviridae, Podoviridae and Siphoviridae, all with double-stranded DNA genomes, as well as the Microviridae family, which possess single-stranded DNA genomes (Manrique et al., 2016; Reyes et al., 2015). As mentioned, the exploitation of virome data has proved more challenging than that of genomic data for other components of the microbiota. Beside viral identification, an even greater hurdle is the lack of a universal tool for matching the predating phages to their host bacteria. Progress in this direction has been made to refine predictions, for example by looking for bacterial CRISPR (clustered regularly interspaced short palindromic repeats) spacer sequences with homology to known viral genome sequences. The technique was successful in identifying the match between phage and bacterial species, the next challenge will be to define this match at the strain level (Paez-Espino et al., 2019). As a result, progress in the role of phages in microbiota has been based mostly on experimental models built from data obtained *in silico*, *in vitro* and *in vivo* (Scanlan, 2017). In this review, we will integrate recent data from phage biology into the broader context of the coevolution of phages and bacteria within the GIT and discuss the possible effects of this coevolution on the human host.

Interactions between phages and bacteria: from test tube to the intestinal organ

Studies of phage-bacterium interactions (PBI) began with the launch of phage therapy a century ago (d'Herelle, 1917). Many original molecular mechanisms affecting these interactions have since been identified, mostly from studies of individual phage-bacterium pairs cultured in optimal laboratory conditions. A broad summary of these mechanisms is presented in Figure 1, and several reviews have described the resistance systems developed by bacteria and the counter-defence strategies used by phages (de Jonge et al., 2018; Labrie et al., 2010). In addition to broad mechanisms, such as alterations to receptor and restriction-modification systems, more specific and novel systems have been recently uncovered by data mining and large-scale screening, and it is thought that many more remain to be discovered (Doron et al., 2018; Kronheim et al., 2018). The flexibility of genetic information forms the cornerstone of all these systems. Therefore, integration of the evolution of PBI in the intestinal context requires the consideration of multiple levels of information, from small viral genomes to the behaviour of large organs.

Evidences for the coevolution of phages and bacteria in the GIT

The coevolution of phages and bacteria gives rise to structured nested and modular networks. Nested interaction networks are characterised by a hierarchy of bacteria and phages ranked according to the susceptibility or resistance of bacteria and to the specialist (infecting few strains) or generalist (infecting many strains) nature of the phages. By contrast, in modular networks, interactions occur within distinct groups of phages and bacteria different from those present in other modules, with very little overlap (Weitz et al., 2013). These two types of interactions may also coexist within the nested-modular networks of complex ecosystems, including the mouse GIT in which generalist phages are prevalent (De Sordi et al., 2017; Kim and Bae, 2018). This level of interaction is subject to dynamic modulation by the evolution of defence and counter-defence systems of bacteria and phages. For example, resistance to phages has been observed in studies characterising clinical samples from *Vibrio cholerae*-infected patients, and phage-treated chickens infected with *Campylobacter jejuni* or calves infected with *Escherichia coli* (Holst Sorensen et al., 2012; Seed et al., 2014; Smith and Huggins, 1983).

Moreover, intestinal metagenomic analyses have revealed the existence of considerable variability in bacterial surface epitopes, including phage receptors, within a given bacterial species isolated from different subjects (Zhu et al., 2015), supporting a hypothesis of active local coevolution between phages and bacteria. A similar conclusion was drawn from the metagenomic detection of highly variable and rapidly evolving CRISPR sequences, suggesting multiple attempts to escape phage predation (Stern et al., 2012). These genomic events do not necessarily give rise to a dominant population of bacteria with a phenotype of phage resistance. Indeed, experimental phage-bacteria coevolution studies in animal models have failed to recover phenotypically resistant bacteria from isolated colonies, and large viromic studies in humans have failed to detect metagenomic signs of coevolution (De Sordi et al., 2018; Minot et al., 2013). Nevertheless, coevolution has persisted over time, as demonstrated by the existence of mutations of bacterial loci relating to phage receptors. This suggests that either phages deploy rapid counter defence mechanisms or that alternative resistance mechanisms operate. This phenotypic resistance of bacteria is conceptually similar to the tolerance to antibiotics and persistence of bacteria in the presence of antibiotics, and may emerge within the intestinal organ (Lourenco et al., 2018).

A complementary hypothesis is that bacteria may display a different physiological state locally, rendering them less permissive to phage infection (Denou et al., 2007). Indeed, bacteria may display differential susceptibility to phages between the niches occupied in the GIT, as shown by replication data obtained *ex vivo*. The CLB_P1 phage is capable of replicating in ileal sections but not in faeces collected from the same mouse colonised by the *E. coli* strain targeted by this phage (Maura et al., 2012). Using the same assay, other phages were independently efficient in other gut sections, demonstrating that the observed phenotypic resistance is phage-specific (Galtier et al., 2016b; Maura et al., 2012). Gradients of abiotic factors, such as pH and oxygen concentration, and of metabolites, such as bile salts and short-chain fatty acids, along the digestive tract can alter the physiology of bacteria and, consequently, their susceptibility to phages. Furthermore, a recent study by Kronheim *et al*., show that bacteria can produce molecules that interfere with the phage infection cycle, revealing an additional source of phenotypic resistance (Kronheim et al., 2018). The GIT is a structured environment with transverse and longitudinal differences in microbial density, and layers of mucus and villi. This spatially heterogeneous organ can provide niches in which coevolution does not occur. As an example, T4 phages have been shown to display differential ability to persist in a model of mucosal layer, depending on the presence or absence of Ig-like domains on the viral capsid, therefore affecting the frequency of encountering their hosts (Barr et al., 2013). In addition, immune cells that are patrolling the human body can also interact with both bacteria and phages, the latter having attracted much less attention from researchers than the first (Van Belleghem et al., 2018). More importantly, each of the above parameters may affect the coevolution of individual pairs of phages and bacteria, highlighting the complexity of studying PBI in the GIT (Figure 2) (Lourenco et al., 2018).

Population dynamics

The antagonistic coevolution of phages and bacteria has an impact on the dynamic fluctuations of both populations. Such dynamics have been described in different models, such as the arms race dynamics (ARD) and the density-dependent fluctuating selection dynamics (FSD) models (Gandon et al., 2008). In the ARD model, both phages and bacteria accumulate genomic mutations, which enable the bacteria to develop resistance and the phages to counteract that resistance, thereby generating predator-prey cycles. Instead, the

158 FSD model is not based on the evolution of phages to overcome bacterial resistance as it
159 takes into account the pleiotropic costs associated with the mutations enabling bacteria to
160 become resistant. Strong predation by phages selects for resistant bacterial populations,
161 thereby decreasing the number of phages present locally. A subsequent absence of phage
162 selective pressure thus favours an expansion of the population of bacteria susceptible to
163 phages, which are not subject to the possible fitness cost associated with the mutations
164 conferring resistance to phages (Hall et al., 2011; Lennon et al., 2007; Middelboe et al.,
165 2009). Conversely, mutations overcoming bacterial resistance may also be a burden to the
166 phage in situations in which such a counter-resistance is not selected. This FSD model applies
167 to both single phage-bacterium pairs and to the heterogeneous populations derived from
168 their coevolution (Breitbart et al., 2018).
169 In any given microbiota in which phages interact with diverse populations of strains,
170 antagonistic coevolution proceeds within a more intricate network of interactions. For
171 example, the antagonistic coevolution of multiple wild marine T7-like cyanophages with
172 their targeted bacteria, *Prochlorococcus*, is characterised by genomic mutations, host range
173 expansion and fitness costs (Enav et al., 2018). The authors confirmed the concomitant
174 occurrence of ARD, shown by the detection of genomic mutations responsible for bacterial
175 resistance and phage re-infectivity, and FSD, due to the genetic hypervariability of the two
176 populations. However, many phage populations did not carry mutations that could
177 overcome *Prochlorococcus* resistance, suggesting that these two coevolutionary models
178 alone cannot account for the maintenance of these phage variants. The authors postulated
179 that host jumps might constitute a third concomitant mechanism based on the selection of
180 genomic mutations in phages that confer an ability to infect alternative bacteria, thereby
181 avoiding the risk of phage extinction and termination of the predator-prey cycle. Host jumps
182 were also reported in a mouse model of coevolution in the GIT (De Sordi et al., 2017). In this
183 model, the *E. coli* phage P10 evolved to infect an initially inaccessible *E. coli* strain during
184 multiple passages in two other *E. coli* strains. The selection of a combination of mutations
185 resulted in greater fitness associated with infection of the inaccessible *E. coli* strain. In these
186 settings, the genomic heterogeneity of both the phage and bacterial populations may also
187 result from simultaneous dynamic population fluctuations and the sustained antagonistic
188 coevolution and generation of community variability (De Sordi et al., 2018).

The application of coevolution models to the intestinal microbiota would theoretically be able to identify a moment in space and time at which the most fit population of phages and bacteria would have the opportunity to replace the most abundant ones. However, from birth to adulthood, the bacterial diversity of the intestinal microbiota is dominated by the same phyla, Bacteroidetes and Firmicutes, to which the most abundant species belong. This observation gave rise to a theoretical royal family model, in which a bacterial population declining after an antagonistic fluctuation is replaced by a related bacterial population, which is already adapted for occupation of the same environmental niche, rather than any other bacteria (Breitbart et al., 2018). This model was first proposed based on analysis of aquatic ecosystems and is supported by the repeated isolation of the same bacterial and viral taxa in parallel antagonistic coevolutions.

A prime example of the dominance of a particular viral group in the GIT is provided by the crAssphage family, the members of which infect bacteria from one of the most widespread phyla, Bacteroiodetes. The crAssphage family was first identified metagenomically in 2014 and was rapidly characterised and found to be widespread, but the first isolated target strain of these phages (*Bacteroiodes intestinalis*) was not identified until 2018, after a laborious search (Dutilh et al., 2014; Shkoporov et al., 2018; Yutin et al., 2018). If that much effort was required to characterise the most abundant antagonistic populations in the human GIT, we can only imagine how difficult the identification of less abundant coevolving pairs of phages and bacteria is likely to be. However, further studies of the evolution of crAssphage and *Bacteroides* populations would provide useful insight into the keys to microbiota stability.

Phage activities related to health and disease

Virulent and temperate phages

It is widely agreed that most phages have the capacity to lyse the bacteria they infect (M13 being a well-known exception relying on chronic infection), and some have in addition a dedicated set of genes (encoding integrase, excisionase, and repressors, for example) required for the integration of their genome into the chromosomes of the bacteria to postpone lysis. These phages are described as “temperate” rather than “virulent” phages, the genomes of virulent phages being devoid of genes encoding such functions. Due to the abundance of integrases on virome analysis, it has been suggested that the majority of phages in the gut would be temperate (Minot et al., 2011; Reyes et al., 2010). The phage

genome integrated into the bacterial chromosome is named prophage. Many sequence analysis tools have been developed for scanning bacterial genomes, and they have revealed that putative prophage sequences can account for up to 20% of the total length of the bacterial genome (Canchaya et al., 2003; Casjens, 2003). The integration of a phage genome is known as lysogenic conversion and has been studied in bacteria for more than 50 years, particularly for the infection of *E. coli* by phage lambda (Lwoff, 1953). This model phage has been studied in detail, and many reports have focused on the conditions governing the excision of the phage lambda genome from the chromosome to re-establish virulent infection. Indeed, several environmental stresses, such as UV light or chemicals (including antibiotics), as well as inflammation in the GIT, can induce prophage excision (Banks et al., 2003; Barnhart et al., 1976; Goerke et al., 2006). Oh et al. recently add dietary fructose and short-chain fatty acids to the list of inducers of prophage excision, suggesting a mechanism of phage-mediated alteration of the intestinal microbiota depending on the bacterial metabolism (Oh et al., 2019). The induction of excision is essential for the perpetuation of temperate phages, as it provides a means of infecting more bacteria and disseminating. The exit of the phage from a bacterial chromosome must, therefore, be precisely controlled by a defined molecular mechanism. This mechanism has been dissected in great detail for phage lambda and is based on a genetic switch governing the production of the CI (lysogeny-promoting) and Cro (excision/lytic-promoting) proteins. Most of the inducers of phage excision provoke DNA damage, triggering an emergency response that is used by the phage to express the genes required for the excision. However, signalling molecules also impact on the decision for excision. A *Vibrio cholerae* phage encodes for a receptor able to activate the phage lytic pathway upon binding to a quorum sensing molecule produced by the bacterial host (Silpe and Bassler, 2018). An alternative system, called arbitrium, has recently been described (Erez et al., 2017). Upon bacterial lysis, a peptide produced by the prophage is released; when the concentration of this peptide in the environment exceeds a particular threshold, the surrounding bacteria perceive the signal (through a set of genes also originating from the phage), leading to the cessation of lysis and the promotion of lysogeny. This novel system is thought to be only one of many original systems awaiting discovery (Howard-Varona et al., 2017). In the confined intestinal environment, where accumulation of small signalling molecules is favoured compared to open environments, such new mechanisms are likely to play a role in shaping the microbial communities. Recent studies on

prophage dynamics in the GIT have shown that prophages can excise from bacterial chromosomes, modulate the microbiome, acquire genetic information or even transfer between bacteria in response to inflammatory processes (Cornuault et al., 2018; De Paepe et al., 2014; De Paepe et al., 2016; Diard et al., 2017; Oh et al., 2019). Overall, in the GIT, the activity of phages, whether temperate or virulent, influences not only phage abundance, but also bacterial behaviour.

Impact of phages on bacterial behaviour and virulence

The lysogenic conversion of bacteria is accompanied by wide-ranging effects on their behaviour. For example, the toxin genes carried by temperate phages, encoding cholera or Shiga toxins can affect bacterial virulence (Bille et al., 2017; Muniesa et al., 2012). Prophage induction in pathogenic bacteria in the GIT may then provide opportunities for the dissemination of such virulence factors. Genes carried by prophages can also influence bacterial physiology by supplying new functions, such as an expansion of metabolic capability conferring an enhanced fitness in this competitive niche (Bille et al., 2017; Brussow et al., 2004; Harrison and Brockhurst, 2017; Obeng et al., 2016). Importantly, prophages, and phages in general, do not usually carry antibiotic resistance genes, suggesting possible counter-selection against such genes within phage genomes (Enault et al., 2017).

In addition to these direct consequences, prophage integration can also lead to indirect effects that are currently underappreciated. For example, prophage integration may change the conformation of the bacterial chromosome, with various effects on gene expression. When the prophage excision mechanism is altered or lost, the genomic information of the phage is locked in the bacterial chromosome, where it is subjected to purifying selection (deletion of deleterious functions) (Bobay et al., 2014). By contrast, lysogenic behaviour is lost on prophage excision and lysis of the host bacterium. Lysogens may then be assimilated as a subpopulation of bacteria with a higher probability of death than non-lysogenic bacteria. On leaving the bacterium, phages may encapsidate some of the genomic information from the bacterium, which may then transduce another bacterium. This property was exploited extensively in the early days of bacterial genetics, with phage P1 the best-known example of a transducing phage. Horizontal gene transfers of this type are now

recognised as a major driving force behind bacterial evolution and adaptation to environmental cues (Howard-Varona et al., 2017).

In the GIT, in which many, if not all, of these events can take place, evidence supporting dynamic prophage induction is emerging. For instance, the cost of carrying a prophage was assessed for *E. coli* in a model of axenic mice colonised by lysogens, and the results revealed a high rate of prophage induction (De Paepe et al., 2016). Induction was also recorded *in vivo* with *Enterococcus faecalis*, and this process was shown to be involved in killing competitors (Duerkop et al., 2012). Studies of this bacterium have also revealed the intricate behaviour of several prophage elements within the same cell: some defective prophages were shown to hijack structural proteins from other intact prophages to form the virions required for their dissemination (Matos et al., 2013). The mammalian host has also recently been considered in studies of the inflammatory response promoting phage transfer from one *Salmonella spp.* strain to another (Diard et al., 2017). This work highlighted the need to take the mammalian host into account in studies of PBI dynamics and vice versa, as well as to study the processes that may lead to alteration in the microbiota associated to infections and inflammatory diseases (Debarbieux, 2014; Galtier et al., 2016a). In addition to the host and its response to the presence of microbes and their dynamics, other factors, such as changes in diet, may affect PBI dynamics by altering metabolic pathways (Oh et al., 2019). Metabolic changes may, in turn, influence the competition between bacteria for particular niches, thereby affecting the mammalian host response and potentially resulting in a shift in the overall stability and evolution of the microbial consortium.

Phages in disease cohorts

Without the advent of metagenomic sequencing, the link between phages and health would probably never have been discovered, because traditional culture methods for viruses are based on the high specificity of PBI. Indeed, the quantification of phages by direct plaquing is restricted by the number of bacterial strains used to perform these tests. This laborious task managed to identify one crAssphage susceptible strain in laboratory conditions only because it was guided by metagenomics information for a faecal sample highly enriched in this family of phages (Shkoporov et al., 2018; Yutin et al., 2018).

One of the earliest studies of the richness and diversity of the phage community associated with changes in intestinal microbiota was performed on faecal samples from patients with

316 Crohn's disease and ulcerative colitis. Surprisingly, both the richness and diversity of phages
317 were higher in these patients than in healthy subjects, but bacterial richness and diversity
318 were lower (Norman et al., 2015). A similar observation was also reported for healthy twins
319 during the first 24 months of life (Reyes et al., 2010). The drivers of these dynamics remain
320 unknown. Do the disease conditions lead to an expansion of the population of prophages
321 excised from low-abundance bacterial populations below the radar of metagenomics
322 analysis? Or do changes in bacterial composition provide newcomers with an opportunity to
323 invade the GIT with the necessary adaptation steps, including shifts in phage populations?
324 The two hypothesis are not to be considered mutually exclusive and methods for full
325 exploitation of the genomic information are still being developed (Roux et al., 2017).
326 However, for the time being, the interpretation of these observations cannot yet extend
327 beyond associations or trends based on the abundance of reads. Nevertheless, increasing
328 examples of changes viral metagenomes are being associated to diseases, like AIDS or
329 diabetes (Manrique et al., 2016; Monaco et al., 2016; Norman et al., 2015; Zhao et al., 2017).
330 Faecal microbiota transfer (FMT) to treat recurrent *Clostridium difficile* infections is a recent
331 development providing support for an active role of phages in shaping the intestinal
332 microbial community. First, viruses from the donor were found to be transferred to the
333 recipient after six weeks of FMT treatment. All the transferred viruses were phages,
334 providing an additional argument in favour of the safety of FMT and the putative role of
335 phages in the success of this treatment (Zuo et al., 2018). A 12-month follow-up study
336 recently showed that phages from the donors were still detectable in the recipients,
337 demonstrating the long-term invasion of the initial microbiota by the phages, and, thus, the
338 ability of these phages to adapt to a different environment (Draper et al., 2018). Moreover,
339 FMT treatment with a sterile filtrate was found to be as effective for reducing *C. difficile*
340 infections as standard FMT (Ott et al., 2017). Overall, these data highlight a major role for
341 phages in the manipulation of the intestinal microbial population. However, it remains
342 undetermined whether phages exert these effects on their own and by which mechanisms
343 they establish and evolve over time.
344
345 **Perspectives**
346 After 100 years of research mostly focused on single phage/bacterium pairs, the field of PBI
347 research is now enjoying a new lease of life, thanks to novel technologies facilitating the

study of complex microbial communities and the need to support phage therapy as one possible solution to the problem of antibiotic resistance (Roach and Debarbieux, 2017). When trying to understand what maintains or disturbs the intestinal microbial balance in healthy subjects, efforts should focus on modelling and defining how different coevolutionary models can concurrently shape microbial diversity in spatially heterogeneous environments (Hannigan et al., 2018). The type and cost of resistance and counter-resistance should be considered, together with the physiological advantages of colonising different niches. The aim is to describe the coexistence of many different phages and bacterial hosts in the microbiota mathematically, and to predict the sweeps likely to alter or maintain the equilibrium in a steady state in the long term. Viral ecologists can provide a framework for achieving this goal, generally by focusing on global ecosystems (Bolduc et al., 2017). The next stage will be to develop models of these coevolution dynamics taking into account the mammalian host in the context of both healthy and diseased subjects (Hochberg, 2018).

Experimental model systems will also be required to decipher individual mechanisms at the molecular level. One-to-one interaction studies can reveal novel ways in which phages and bacteria can manipulate each other's evolution, but access to the mammalian environment remains limited. However, gnotobiotic murine models — germ-free mice colonised by a defined set of bacterial strains —, are emerging as a surrogate system in which the impact of the intestinal microbiota on health can be investigated. Germ-free mice colonised with human bacterial strains have been used to describe phages from human viral faecal material and could be further developed for the isolation of human-associated phages (Reyes et al., 2013). Another model was recently established with murine bacterial strains, providing a more natural intestinal ecosystem compared to introducing human bacterial strains into germ-free mice. This model was then used to demonstrate the basis of the microbial competition between *Salmonella typhimurium* and *E. coli* strains for the same niche (Brugiroux et al., 2016). Such a model has the advantage of high reproducibility between breeding facilities, making it possible for multiple teams with specific objectives to work with the same tool. Murine models also provide biological samples that can be difficult to obtain from humans, such as intestinal biopsy specimens, which are required to assess the influence of spatial distribution on the interaction and evolution of microbial species. Last, but not least, beyond PBI, diverse microbial interactions, such as those between fungi and

bacteria, are currently underappreciated together with interactions amongst other GIT inhabitants, such as enteric parasites.

Finally, an area still overshadowed by the work on PBI and evolution is the effect of the mammalian response to this dynamic consortium of microbes (Figure 3). Disease states, such as inflammation in particular, have been shown to affect PBI, but many other processes remain to be studied in the GIT and in human-associated microbiota in general (Diard et al., 2017; Norman et al., 2015). Following on from the unexpected discovery that relationships between microbiota and drugs can influence the efficacy of immunotherapy against tumors, we can reasonably assume that some PBI play a role on biological processes beyond microbiology (Routy et al., 2018). It remains unclear whether such effects are driven by the antagonistic coevolution of phages and bacteria or a subtler diversion of function exploited by the immune system, but these possibilities highlight how exciting phage biology has become, as it enters a new era of full integration into studies of microbes and their interactions within ecosystems.

Figure legends

Figure 1. Bacterial mechanisms of defence against phage predation. No single bacterium has been found to possess all of these defence systems, but each bacterium can have several. A large pink star indicates the essential mechanisms of DNA replication, transcription and translation underlying the genetic and phenotypic variations inherent to life. Red crosses correspond to an arrest of the infection process. Red triangles correspond to both bacterial proteins involved in the abortive infection (Abi) system leading to cell suicide, and phage proteins involved in the super-infection exclusion (Sie) mechanism to prevent further infection by related phages. Blue and green DNA molecules correspond to bacterial and phage DNA, respectively. R-M, restriction-modification.

Figure 2. Factors influencing phage-bacteria interactions in the gastrointestinal tract. In healthy subjects, abiotic and biotic factors can affect bacteria gene expression (rods in different shades of blue) or mammalian cells, with direct consequences for phage populations. Microbiota in Crohn's disease patients is characterised by an inverse correlation between phage and bacterial diversities (decrease of different coloured rods and increase of phages with various colours). Intestinal villi can serve as spatial refuges for bacteria, enabling them to escape phage predation, represented by dashed lines and multiple phages. Epithelial cells and the mucus layer are coloured in rose and yellow, respectively, with the rare goblet and Paneth cells noted. B, B cells; DC, dendritic cells; M, macrophages; N, neutrophils; T, T cells.

Figure 3. Schematic diagram of the antagonistic coevolution of phages and bacteria in a mammalian host. Heterogeneous populations of bacteria (differentially coloured rods) with different phenotypes (distinct shading within clusters) coexist with various phages (different colours are consistent with bacterial diversity and distinct shades of colours correspond to evolved phages including host jumps). The infection of bacteria by phages affects the fitness of both phage and bacterial populations within a defined spatial niche that is represented by an assemblage of bacteria either coloured in blue (corresponding to phage-resistant/inaccessible populations), orange (corresponding to bacteria lysed by virulent phages, dashed lines) or green (corresponding to lysogens from which prophage [red circles]

excise). The mammalian host (represented as a pink area) underlies these microbial interactions and can modulate and be modulated by the outcome of these interactions.

References

Banks, D.J., Lei, B., and Musser, J.M. (2003). Prophage induction and expression of prophage-encoded virulence factors in group A Streptococcus serotype M3 strain MGAS315. Infection and immunity *71*, 7079-7086.

Barnhart, B.J., Cox, S.H., and Jett, J.H. (1976). Prophage induction and inactivation by UV light. Journal of virology *18*, 950-955.

Barr, J.J., Auro, R., Furlan, M., Whiteson, K.L., Erb, M.L., Pogliano, J., Stotland, A., Wolkowicz, R., Cutting, A.S., Doran, K.S*., et al.* (2013). Bacteriophage adhering to mucus provide a non-host-derived immunity. Proc Natl Acad Sci U S A *110*, 10771-10776.

Belkaid, Y., and Hand, T.W. (2014). Role of the microbiota in immunity and inflammation. Cell *157*, 121-141.

Bille, E., Meyer, J., Jamet, A., Euphrasie, D., Barnier, J.P., Brissac, T., Larsen, A., Pelissier, P., and Nassif, X. (2017). A virulence-associated filamentous bacteriophage of Neisseria meningitidis increases host-cell colonisation. PLoS pathogens *13*, e1006495.

Bobay, L.M., Touchon, M., and Rocha, E.P. (2014). Pervasive domestication of defective prophages by bacteria. Proc Natl Acad Sci U S A *111*, 12127-12132.

Bolduc, B., Youens-Clark, K., Roux, S., Hurwitz, B.L., and Sullivan, M.B. (2017). iVirus: facilitating new insights in viral ecology with software and community data sets imbedded in a cyberinfrastructure. The ISME journal *11*, 7-14.

Breitbart, M., Bonnain, C., Malki, K., and Sawaya, N.A. (2018). Phage puppet masters of the marine microbial realm. Nature microbiology *3*, 754-766.

Brugiroux, S., Beutler, M., Pfann, C., Garzetti, D., Ruscheweyh, H.J., Ring, D., Diehl, M., Herp, S., Lotscher, Y., Hussain, S*., et al.* (2016). Genome-guided design of a defined mouse microbiota that confers colonization resistance against Salmonella enterica serovar Typhimurium. Nature microbiology *2*, 16215.

Brussow, H., Canchaya, C., and Hardt, W.D. (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiology and molecular biology reviews : MMBR *68*, 560-602, table of contents.

Canchaya, C., Proux, C., Fournous, G., Bruttin, A., and Brussow, H. (2003). Prophage genomics. Microbiology and molecular biology reviews : MMBR *67*, 238-276, table of contents.

Casjens, S. (2003). Prophages and bacterial genomics: what have we learned so far? Molecular microbiology *49*, 277-300.

Cornuault, J.K., Petit, M.A., Mariadassou, M., Benevides, L., Moncaut, E., Langella, P., Sokol, H., and De Paepe, M. (2018). Phages infecting Faecalibacterium prausnitzii belong to novel viral genera that help to decipher intestinal viromes. Microbiome *6*, 65.

d'Herelle, F. (1917). Sur un microbe invisible antagoniste des bacilles dysentériques. Les Comptes rendus de l'Académie des sciences *165*, 373-375.

de Jonge, P.A., Nobrega, F.L., Brouns, S.J.J., and Dutilh, B.E. (2018). Molecular and Evolutionary Determinants of Bacteriophage Host Range. Trends Microbiol.

De Paepe, M., Hutinet, G., Son, O., Amarir-Bouhram, J., Schbath, S., and Petit, M.A. (2014). Temperate phages acquire DNA from defective prophages by relaxed homologous recombination: the role of Rad52-like recombinases. PLoS genetics *10*, e1004181.

De Paepe, M., Tournier, L., Moncaut, E., Son, O., Langella, P., and Petit, M.A. (2016). Carriage of lambda Latent Virus Is Costly for Its Bacterial Host due to Frequent Reactivation in Monoxenic Mouse Intestine. PLoS genetics *12*, e1005861.

De Sordi, L., Khanna, V., and Debarbieux, L. (2017). The Gut Microbiota Facilitates Drifts in the Genetic Diversity and Infectivity of Bacterial Viruses. Cell host & microbe *22*, 801-808.e803.

De Sordi, L., Lourenço, M., and Debarbieux, L. (2018). "I will survive": A tale of bacteriophage-bacteria coevolution in the gut. Gut Microbes, 1-8.

Debarbieux, L. (2014). Bacterial sensing of bacteriophages in communities: the search for the Rosetta stone. Current opinion in microbiology *20*, 125-130.

Denou, E., Berger, B., Barretto, C., Panoff, J.M., Arigoni, F., and Brussow, H. (2007). Gene expression of commensal Lactobacillus johnsonii strain NCC533 during in vitro growth and in the murine gut. Journal of bacteriology *189*, 8109-8119.
Diard, M., Bakkeren, E., Cornuault, J.K., Moor, K., Hausmann, A., Sellin, M.E., Loverdo, C., Aertsen, A., Ackermann, M., De Paepe, M.*, et al.* (2017). Inflammation boosts bacteriophage transfer between Salmonella spp. Science (New York, NY) *355*, 1211-1215.
Doron, S., Melamed, S., Ofir, G., Leavitt, A., Lopatina, A., Keren, M., Amitai, G., and Sorek, R. (2018). Systematic discovery of antiphage defense systems in the microbial pangenome. Science (New York, NY).
Draper, L.A., Ryan, F.J., Smith, M.K., Jalanka, J., Mattila, E., Arkkila, P.A., Ross, R.P., Satokari, R., and Hill, C. (2018). Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation. Microbiome *6*, 220.
Duerkop, B.A., Clements, C.V., Rollins, D., Rodrigues, J.L., and Hooper, L.V. (2012). A composite bacteriophage alters colonization by an intestinal commensal bacterium. Proc Natl Acad Sci U S A *109*, 17621-17626.
Dutilh, B.E., Cassman, N., McNair, K., Sanchez, S.E., Silva, G.G., Boling, L., Barr, J.J., Speth, D.R., Seguritan, V., Aziz, R.K.*, et al.* (2014). A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes. Nature communications *5*, 4498.
Enault, F., Briet, A., Bouteille, L., Roux, S., Sullivan, M.B., and Petit, M.A. (2017). Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. The ISME journal *11*, 237-247.
Enav, H., Kirzner, S., Lindell, D., Mandel-Gutfreund, Y., and Beja, O. (2018). Adaptation to sub-optimal hosts is a driver of viral diversification in the ocean. bioRxiv.
Erez, Z., Steinberger-Levy, I., Shamir, M., Doron, S., Stokar-Avihail, A., Peleg, Y., Melamed, S., Leavitt, A., Savidor, A., Albeck, S.*, et al.* (2017). Communication between viruses guides lysis-lysogeny decisions. Nature *541*, 488-493.
Faruque, S.M., Naser, I.B., Islam, M.J., Faruque, A.S., Ghosh, A.N., Nair, G.B., Sack, D.A., and Mekalanos, J.J. (2005). Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. Proc Natl Acad Sci U S A *102*, 1702-1707.
Frank, D.N., St Amand, A.L., Feldman, R.A., Boedeker, E.C., Harpaz, N., and Pace, N.R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A *104*, 13780-13785.
Galtier, M., De Sordi, L., Maura, D., Arachchi, H., Volant, S., Dillies, M.A., and Debarbieux, L. (2016a). Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition. Environmental microbiology *18*, 2237-2245.
Galtier, M., De Sordi, L., Neut, C., and Debarbieux, L. (2016b). Bacteriophages targeting adherent invasive Escherichia coli strains as a promising new treatment for Crohn's disease. J Crohns Colitis.
Gandon, S., Buckling, A., Decaestecker, E., and Day, T. (2008). Host-parasite coevolution and patterns of adaptation across time and space. J Evol Biol *21*, 1861-1866.
Gentile, C.L., and Weir, T.L. (2018). The gut microbiota at the intersection of diet and human health. Science (New York, NY) *362*, 776-780.
Goerke, C., Koller, J., and Wolz, C. (2006). Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in Staphylococcus aureus. Antimicrob Agents Chemother *50*, 171-177.
Hall, A.R., Scanlan, P.D., Morgan, A.D., and Buckling, A. (2011). Host-parasite coevolutionary arms races give way to fluctuating selection. Ecology letters *14*, 635-642.
Hannigan, G.D., Duhaime, M.B., Koutra, D., and Schloss, P.D. (2018). Biogeography and environmental conditions shape bacteriophage-bacteria networks across the human microbiome. PLoS computational biology *14*, e1006099.
Harrison, E., and Brockhurst, M.A. (2017). Ecological and Evolutionary Benefits of Temperate Phage: What Does or Doesn't Kill You Makes You Stronger. BioEssays : news and reviews in molecular, cellular and developmental biology *39*.
Hochberg, M.E. (2018). An ecosystem framework for understanding and treating disease. Evol Med Public Health *2018*, 270-286.

Holst Sorensen, M.C., van Alphen, L.B., Fodor, C., Crowley, S.M., Christensen, B.B., Szymanski, C.M., and Brondsted, L. (2012). Phase variable expression of capsular polysaccharide modifications allows Campylobacter jejuni to avoid bacteriophage infection in chickens. Frontiers in cellular and infection microbiology *2*, 11.
Howard-Varona, C., Hargreaves, K.R., Abedon, S.T., and Sullivan, M.B. (2017). Lysogeny in nature: mechanisms, impact and ecology of temperate phages. The ISME journal *11*, 1511-1520.
Kim, M.S., and Bae, J.W. (2018). Lysogeny is prevalent and widely distributed in the murine gut microbiota. The ISME journal *12*, 1127-1141.
Koskella, B., and Brockhurst, M.A. (2014). Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. FEMS Microbiol Rev *38*, 916-931.
Kronheim, S., Daniel-Ivad, M., Duan, Z., Hwang, S., Wong, A.I., Mantel, I., Nodwell, J.R., and Maxwell, K.L. (2018). A chemical defence against phage infection. Nature *564*, 283-286.
Labrie, S.J., Samson, J.E., and Moineau, S. (2010). Bacteriophage resistance mechanisms. Nat Rev Microbiol *8*, 317-327.
Lennon, J.T., Khatana, S.A., Marston, M.F., and Martiny, J.B. (2007). Is there a cost of virus resistance in marine cyanobacteria? The ISME journal *1*, 300-312.
Ley, R.E., Turnbaugh, P.J., Klein, S., and Gordon, J.I. (2006). Microbial ecology: human gut microbes associated with obesity. Nature *444*, 1022-1023.
Lourenco, M., De Sordi, L., and Debarbieux, L. (2018). The Diversity of Bacterial Lifestyles Hampers Bacteriophage Tenacity. Viruses *10*.
Lwoff, A. (1953). Lysogeny. Bacteriological reviews *17*, 269-337.
Manrique, P., Bolduc, B., Walk, S.T., van der Oost, J., de Vos, W.M., and Young, M.J. (2016). Healthy human gut phageome. Proc Natl Acad Sci U S A *113*, 10400-10405.
Matos, R.C., Lapaque, N., Rigottier-Gois, L., Debarbieux, L., Meylheuc, T., Gonzalez-Zorn, B., Repoila, F., Lopes Mde, F., and Serror, P. (2013). Enterococcus faecalis prophage dynamics and contributions to pathogenic traits. PLoS genetics *9*, e1003539.
Maura, D., Galtier, M., Le Bouguenec, C., and Debarbieux, L. (2012). Virulent bacteriophages can target O104:H4 enteroaggregative Escherichia coli in the mouse intestine. Antimicrob Agents Chemother *56*, 6235-6242.
Middelboe, M., Holmfeldt, K., Riemann, L., Nybroe, O., and Haaber, J. (2009). Bacteriophages drive strain diversification in a marine Flavobacterium: implications for phage resistance and physiological properties. Environmental microbiology *11*, 1971-1982.
Minot, S., Bryson, A., Chehoud, C., Wu, G.D., Lewis, J.D., and Bushman, F.D. (2013). Rapid evolution of the human gut virome. Proc Natl Acad Sci U S A *110*, 12450-12455.
Minot, S., Sinha, R., Chen, J., Li, H., Keilbaugh, S.A., Wu, G.D., Lewis, J.D., and Bushman, F.D. (2011). The human gut virome: inter-individual variation and dynamic response to diet. Genome research *21*, 1616-1625.
Monaco, C.L., Gootenberg, D.B., Zhao, G., Handley, S.A., Ghebremichael, M.S., Lim, E.S., Lankowski, A., Baldridge, M.T., Wilen, C.B., Flagg, M.*, et al.* (2016). Altered Virome and Bacterial Microbiome in Human Immunodeficiency Virus-Associated Acquired Immunodeficiency Syndrome. Cell host & microbe *19*, 311-322.
Muniesa, M., Hammerl, J.A., Hertwig, S., Appel, B., and Brussow, H. (2012). Shiga toxin-producing Escherichia coli O104:H4: a new challenge for microbiology. Applied and environmental microbiology *78*, 4065-4073.
Norman, J.M., Handley, S.A., Baldridge, M.T., Droit, L., Liu, C.Y., Keller, B.C., Kambal, A., Monaco, C.L., Zhao, G., Fleshner, P.*, et al.* (2015). Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell *160*, 447-460.
Obeng, N., Pratama, A.A., and Elsas, J.D.V. (2016). The Significance of Mutualistic Phages for Bacterial Ecology and Evolution. Trends Microbiol *24*, 440-449.
Oh, J., Alexander, L., Pan, M., Schueler, K., Keller, M., Attie, A., Walter, J., and van Pijkeren, J. (2019). Dietary Fructose and Microbiota-Derived Short-Chain Fatty Acids Promote Bacteriophage Production in the Gut Symbiont Lactobacillus reuteri. Cell host & microbe *25*, in press.

583 Ott, S.J., Waetzig, G.H., Rehman, A., Moltzau-Anderson, J., Bharti, R., Grasis, J.A., Cassidy, L., Tholey,
584 A., Fickenscher, H., Seegert, D.*, et al.* (2017). Efficacy of Sterile Fecal Filtrate Transfer for Treating
585 Patients With Clostridium difficile Infection. Gastroenterology *152*, 799-811 e797.
586 Paez-Espino, D., Eloe-Fadrosh, E.A., Pavlopoulos, G.A., Thomas, A.D., Huntemann, M., Mikhailova, N.,
587 Rubin, E., Ivanova, N.N., and Kyrpides, N.C. (2016). Uncovering Earth's virome. Nature *536*, 425-430.
588 Paez-Espino, D., Roux, S., Chen, I.A., Palaniappan, K., Ratner, A., Chu, K., Huntemann, M., Reddy,
589 T.B.K., Pons, J.C., Llabres, M.*, et al.* (2019). IMG/VR v.2.0: an integrated data management and
590 analysis system for cultivated and environmental viral genomes. Nucleic Acids Res *47*, D678-D686.
591 Pasolli, E., Asnicar, F., Manara, S., Zolfo, M., Karcher, N., Armanini, F., Beghini, F., Manghi, P., Tett, A.,
592 Ghensi, P.*, et al.* (2019). Extensive Unexplored Human Microbiome Diversity Revealed by Over
593 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle. Cell.
594 Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D.*, et al.* (2012). A
595 metagenome-wide association study of gut microbiota in type 2 diabetes. Nature *490*, 55-60.
596 Reyes, A., Blanton, L.V., Cao, S., Zhao, G., Manary, M., Trehan, I., Smith, M.I., Wang, D., Virgin, H.W.,
597 Rohwer, F.*, et al.* (2015). Gut DNA viromes of Malawian twins discordant for severe acute
598 malnutrition. Proc Natl Acad Sci U S A *112*, 11941-11946.
599 Reyes, A., Haynes, M., Hanson, N., Angly, F.E., Heath, A.C., Rohwer, F., and Gordon, J.I. (2010).
600 Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature *466*, 334-338.
601 Reyes, A., Semenkovich, N.P., Whiteson, K., Rohwer, F., and Gordon, J.I. (2012). Going viral: next-
602 generation sequencing applied to phage populations in the human gut. Nat Rev Microbiol *10*, 607-
603 617.
604 Reyes, A., Wu, M., McNulty, N.P., Rohwer, F.L., and Gordon, J.I. (2013). Gnotobiotic mouse model of
605 phage-bacterial host dynamics in the human gut. Proc Natl Acad Sci U S A *110*, 20236-20241.
606 Roach, D.R., and Debarbieux, L. (2017). Phage therapy: awakening a sleeping giant. Emerging Topics
607 in Life Sciences *1*, 93-103.
608 Routy, B., Le Chatelier, E., Derosa, L., Duong, C.P.M., Alou, M.T., Daillere, R., Fluckiger, A.,
609 Messaoudene, M., Rauber, C., Roberti, M.P.*, et al.* (2018). Gut microbiome influences efficacy of PD-
610 1-based immunotherapy against epithelial tumors. Science (New York, NY) *359*, 91-97.
611 Roux, S., Emerson, J.B., Eloe-Fadrosh, E.A., and Sullivan, M.B. (2017). Benchmarking viromics: an in
612 silico evaluation of metagenome-enabled estimates of viral community composition and diversity.
613 PeerJ *5*, e3817.
614 Scanlan, P.D. (2017). Bacteria-Bacteriophage Coevolution in the Human Gut: Implications for
615 Microbial Diversity and Functionality. Trends Microbiol.
616 Seed, K.D., Yen, M., Shapiro, B.J., Hilaire, I.J., Charles, R.C., Teng, J.E., Ivers, L.C., Boncy, J., Harris, J.B.,
617 and Camilli, A. (2014). Evolutionary consequences of intra-patient phage predation on microbial
618 populations. eLife *3*, e03497.
619 Sharon, G., Sampson, T.R., Geschwind, D.H., and Mazmanian, S.K. (2016). The Central Nervous
620 System and the Gut Microbiome. Cell *167*, 915-932.
621 Shkoporov, A.N., Khokhlova, E.V., Fitzgerald, C.B., Stockdale, S.R., Draper, L.A., Ross, R.P., and Hill, C.
622 (2018). PhiCrAss001 represents the most abundant bacteriophage family in the human gut and
623 infects Bacteroides intestinalis. Nature communications *9*, 4781.
624 Silpe, J.E., and Bassler, B.L. (2018). A Host-Produced Quorum-Sensing Autoinducer Controls a Phage
625 Lysis-Lysogeny Decision. Cell.
626 Singer, E., Wagner, M., and Woyke, T. (2017). Capturing the genetic makeup of the active
627 microbiome in situ. The ISME journal *11*, 1949-1963.
628 Smith, H.W., and Huggins, M.B. (1983). Effectiveness of phages in treating experimental Escherichia
629 coli diarrhoea in calves, piglets and lambs. J Gen Microbiol *129*, 2659-2675.
630 Sonnenburg, J.L., Xu, J., Leip, D.D., Chen, C.H., Westover, B.P., Weatherford, J., Buhler, J.D., and
631 Gordon, J.I. (2005). Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science (New
632 York, NY) *307*, 1955-1959.
633 Stern, A., Mick, E., Tirosh, I., Sagy, O., and Sorek, R. (2012). CRISPR targeting reveals a reservoir of
634 common phages associated with the human gut microbiome. Genome research *22*, 1985-1994.

635 Van Belleghem, J.D., Dabrowska, K., Vaneechoutte, M., Barr, J.J., and Bollyky, P.L. (2018). Interactions
636 between Bacteriophage, Bacteria, and the Mammalian Immune System. Viruses *11*.
637 Weitz, J.S., Poisot, T., Meyer, J.R., Flores, C.O., Valverde, S., Sullivan, M.B., and Hochberg, M.E.
638 (2013). Phage-bacteria infection networks. Trends Microbiol *21*, 82-91.
639 Yutin, N., Makarova, K.S., Gussow, A.B., Krupovic, M., Segall, A., Edwards, R.A., and Koonin, E.V.
640 (2018). Discovery of an expansive bacteriophage family that includes the most abundant viruses from
641 the human gut. Nature microbiology *3*, 38-46.
642 Zhao, G., Vatanen, T., Droit, L., Park, A., Kostic, A.D., Poon, T.W., Vlamakis, H., Siljander, H.,
643 Harkonen, T., Hamalainen, A.M.*, et al.* (2017). Intestinal virome changes precede autoimmunity in
644 type I diabetes-susceptible children. Proc Natl Acad Sci U S A *114*, E6166-E6175.
645 Zhu, A., Sunagawa, S., Mende, D.R., and Bork, P. (2015). Inter-individual differences in the gene
646 content of human gut bacterial species. Genome biology *16*, 82.
647 Zhu, X., Han, Y., Du, J., Liu, R., Jin, K., and Yi, W. (2017). Microbiota-gut-brain axis and the central
648 nervous system. Oncotarget *8*, 53829-53838.
649 Zuo, T., Wong, S.H., Lam, K., Lui, R., Cheung, K., Tang, W., Ching, J.Y.L., Chan, P.K.S., Chan, M.C.W.,
650 Wu, J.C.Y.*, et al.* (2018). Bacteriophage transfer during faecal microbiota transplantation in
651 Clostridium difficile infection is associated with treatment outcome. Gut *67*, 634-643.

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Figure 1

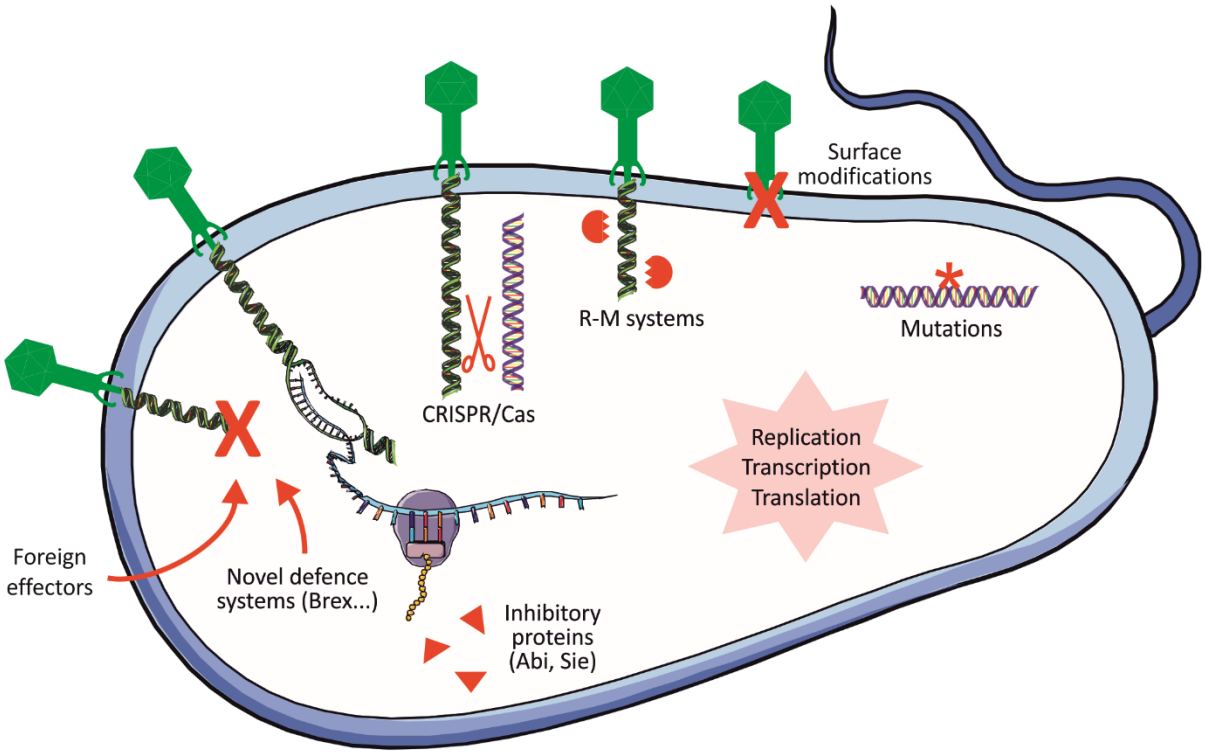

Figure 1. Bacterial mechanisms of defence against phage predation. No single bacterium has been found to possess all of these defence systems, but each bacterium can have several. A large pink star indicates the essential mechanisms of DNA replication, transcription and translation underlying the genetic and phenotypic variations inherent to life. Red crosses correspond to an arrest of the infection process. Red triangles correspond to both bacterial proteins involved in the abortive infection (Abi) system leading to cell suicide, and phage proteins involved in the super-infection exclusion (Sie) mechanism to prevent further infection by related phages. Blue and green DNA molecules correspond to bacterial and phage DNA, respectively. R-M, restriction-modification.

Figure 2

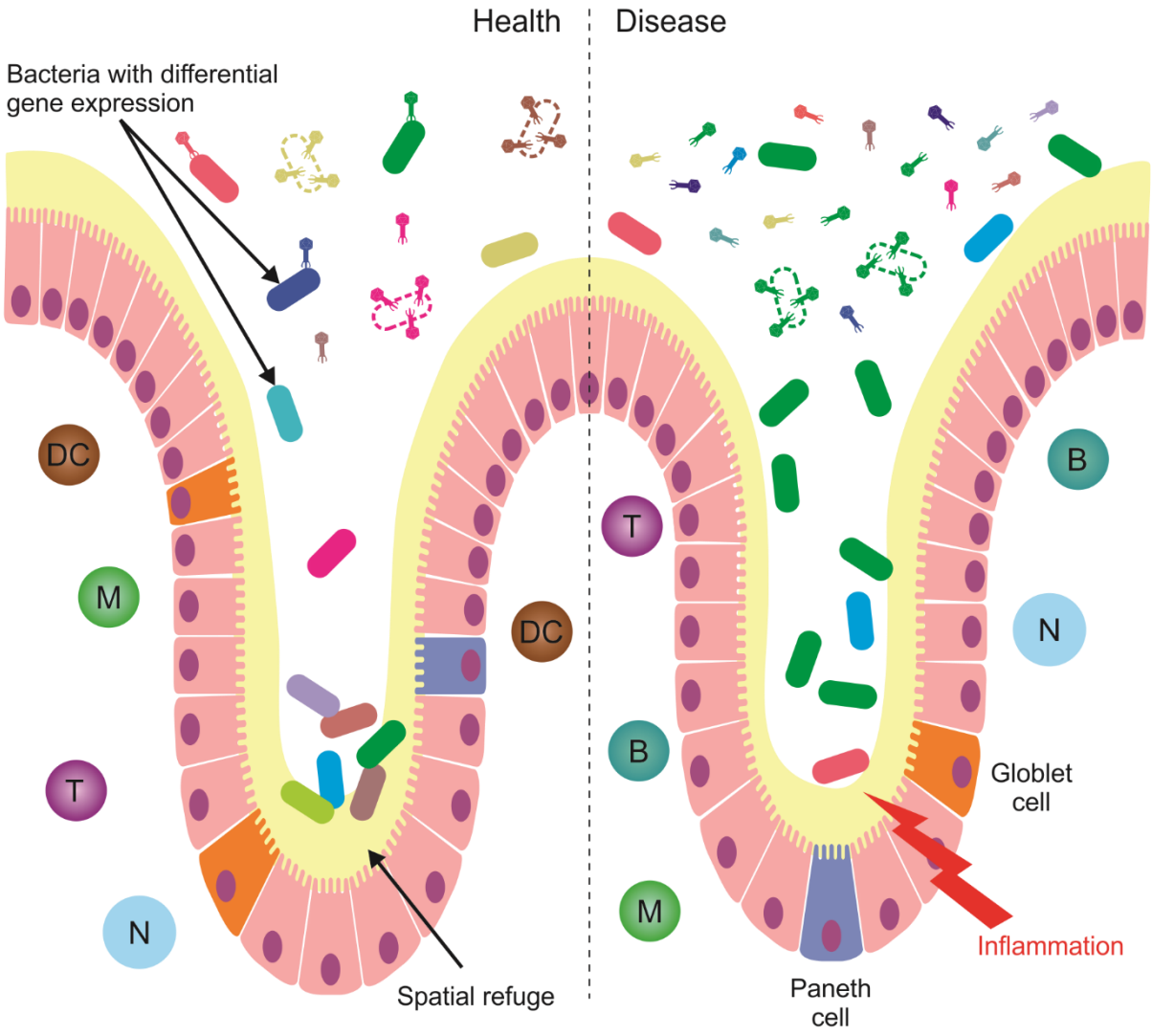

Figure 2. Factors influencing phage-bacteria interactions in the gastrointestinal tract. In healthy individuals, abiotic and biotic factors can affect bacteria gene expression (rods in different shades of blue) or mammalian cells, with direct consequences for phage populations. Microbiota in Crohn's disease patients is characterised by an inverse correlation between phage and bacterial diversities (decrease of different coloured rods and increase of phages with various colours). Intestinal villi can serve as spatial refuges for bacteria, enabling them to escape phage predation, represented by dashed lines and multiple phages. Epithelial cells and the mucus layer are coloured in rose and yellow, respectively, with the rare goblet and Paneth cells noted. B, B cells; DC, dendritic cells; M, macrophages; N, neutrophils; T, T cells.

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Figure 3

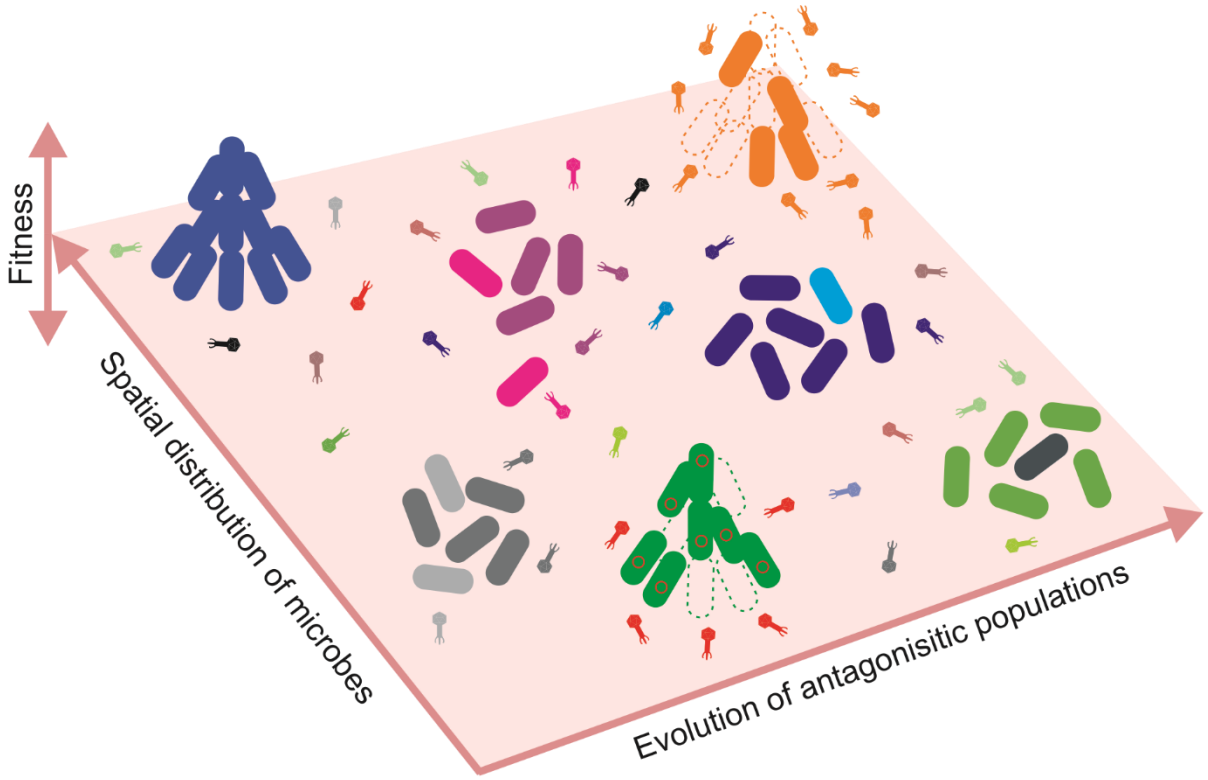

Figure 3. Schematic diagram of the antagonistic coevolution of phages and bacteria in a mammalian host. Heterogeneous populations of bacteria (differentially coloured rods) with different phenotypes (distinct shading within clusters) coexist with various phages (different colours are consistent with bacterial diversity and distinct shades of colours correspond to evolved phages including host jumps). The infection of bacteria by phages affects the fitness of both phage and bacterial populations within a defined spatial niche that is represented by an assemblage of bacteria either coloured in blue (corresponding to phage-resistant/inaccessible populations), orange (corresponding to bacteria lysed by virulent phages, dashed lines) or green (corresponding to lysogens from which prophage [red circles] excise). The mammalian host (represented as a pink area) underlies these microbial interactions and can modulate and be modulated by the outcome of these interactions.