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

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1 Title:

2 The battle within: interactions of bacteriophages and bacteria in the gastrointestinal tract

3

4 Authors:

5 Luisa De Sordi^{1,2}, Marta Lourenço^{1,3}, Laurent Debarbieux^{1*}

6

7 Affiliations:

8 1 Department of Microbiology, Institut Pasteur, Paris F-75015 France.

9 2 Sorbonne Université, UMRS938 INSERM Centre de Recherche Saint-Antoine, Paris, France

10 3 Sorbonne Université, Collège Doctoral, F-75005 Paris, France.

11

12 * Correspondence: laurent.debarbieux@pasteur.fr

13

14 Lead contact: Laurent Debarbieux

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16

17 **Abstract**

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

29

30 **Introduction**

31 Polymicrobial communities, hereafter termed microbiota, are present in most environments
32 and shape the ecology of these environments through metabolic reactions vital to
33 ecosystem function. Within such communities, microbes can establish interspecies networks
34 that can coordinate energetic pathways with effects on the environment at the global scale,
35 as illustrated by the biogeochemical cycling processes that occur in bodies of water and the
36 soil (Singer et al., 2017). Several microbiota also establish intimate associations with
37 multicellular organisms, such as plants or animals, sometimes causing disease but more
38 often developing a symbiotic coexistence of mutual benefit. The human intestinal microbiota
39 is an example of such mutualism that is currently extensively studied, being associated with
40 vital functions, such as digestion, the immune response and the nervous system (Belkaid and
41 Hand, 2014; Sharon et al., 2016; Sonnenburg et al., 2005). Analyses of clinical samples have
42 revealed that several diseases and disorders are associated with alterations in the
43 composition of the intestinal microbiota compared to controls (Frank et al., 2007; Ley et al.,
44 2006; Qin et al., 2012; Zhu et al., 2017). This active field of research is currently focusing on
45 delineating the role of individual components of the microbiota, with the goal to re-establish
46 health (Gentile and Weir, 2018).

47 The intestinal microbiota consist of bacteria, archaea, fungi, protists and viruses. The advent
48 of high-throughput sequencing has opened to the door to revealing the very large microbial
49 diversity associated to the intestinal ecosystem, which is further expanding as new cohorts
50 are analysed worldwide (Pasolli et al., 2019). However, not all the genomic information can
51 be assigned to a defined organism, and this is particularly true for the identification of
52 viruses, at least in part because of the lack of common genomic markers, such as the 16S or
53 18S ribosomal RNA genes (Paez-Espino et al., 2016). Parasitic interactions occur within the
54 gastrointestinal tract (GIT), as exemplified by the most abundant microbes in this
55 environment: bacteria and the viruses that predate on them, bacteriophages (phages). The
56 perpetuating antagonistic coevolution between the predator (phages) and the prey
57 (bacteria) populations results in fluctuations of both these populations (Faruque et al., 2005;
58 Koskella and Brockhurst, 2014).

59 The presence of viruses, including phages in particular, in the human GIT, has been known
60 for a century, but their role in the intestinal microbiota has been little studied (d'Herelle,
61 1917; Reyes et al., 2012). The number of active phage species in a healthy subject has been

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

79

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

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

95 *Evidences for the coevolution of phages and bacteria in the GIT*

96 The coevolution of phages and bacteria gives rise to structured nested and modular
97 networks. Nested interaction networks are characterised by a hierarchy of bacteria and
98 phages ranked according to the susceptibility or resistance of bacteria and to the specialist
99 (infecting few strains) or generalist (infecting many strains) nature of the phages. By
100 contrast, in modular networks, interactions occur within distinct groups of phages and
101 bacteria different from those present in other modules, with very little overlap (Weitz et al.,
102 2013). These two types of interactions may also coexist within the nested-modular networks
103 of complex ecosystems, including the mouse GIT in which generalist phages are prevalent
104 (De Sordi et al., 2017; Kim and Bae, 2018). This level of interaction is subject to dynamic
105 modulation by the evolution of defence and counter-defence systems of bacteria and
106 phages. For example, resistance to phages has been observed in studies characterising
107 clinical samples from *Vibrio cholerae*-infected patients, and phage-treated chickens infected
108 with *Campylobacter jejuni* or calves infected with *Escherichia coli* (Holst Sorensen et al.,
109 2012; Seed et al., 2014; Smith and Huggins, 1983).

110 Moreover, intestinal metagenomic analyses have revealed the existence of considerable
111 variability in bacterial surface epitopes, including phage receptors, within a given bacterial
112 species isolated from different subjects (Zhu et al., 2015), supporting a hypothesis of active
113 local coevolution between phages and bacteria. A similar conclusion was drawn from the
114 metagenomic detection of highly variable and rapidly evolving CRISPR sequences, suggesting
115 multiple attempts to escape phage predation (Stern et al., 2012). These genomic events do
116 not necessarily give rise to a dominant population of bacteria with a phenotype of phage
117 resistance. Indeed, experimental phage-bacteria coevolution studies in animal models have
118 failed to recover phenotypically resistant bacteria from isolated colonies, and large viromic
119 studies in humans have failed to detect metagenomic signs of coevolution (De Sordi et al.,
120 2018; Minot et al., 2013). Nevertheless, coevolution has persisted over time, as
121 demonstrated by the existence of mutations of bacterial loci relating to phage receptors.
122 This suggests that either phages deploy rapid counter defence mechanisms or that
123 alternative resistance mechanisms operate. This phenotypic resistance of bacteria is
124 conceptually similar to the tolerance to antibiotics and persistence of bacteria in the
125 presence of antibiotics, and may emerge within the intestinal organ (Lourenco et al., 2018).

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

150

151 *Population dynamics*

152 The antagonistic coevolution of phages and bacteria has an impact on the dynamic
153 fluctuations of both populations. Such dynamics have been described in different models,
154 such as the arms race dynamics (ARD) and the density-dependent fluctuating selection
155 dynamics (FSD) models (Gandon et al., 2008). In the ARD model, both phages and bacteria
156 accumulate genomic mutations, which enable the bacteria to develop resistance and the
157 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).

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

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

210

211 **Phage activities related to health and disease**

212 *Virulent and temperate phages*

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

221 genome integrated into the bacterial chromosome is named prophage. Many sequence
222 analysis tools have been developed for scanning bacterial genomes, and they have revealed
223 that putative prophage sequences can account for up to 20% of the total length of the
224 bacterial genome (Canchaya et al., 2003; Casjens, 2003). The integration of a phage genome
225 is known as lysogenic conversion and has been studied in bacteria for more than 50 years,
226 particularly for the infection of *E. coli* by phage lambda (Lwoff, 1953). This model phage has
227 been studied in detail, and many reports have focused on the conditions governing the
228 excision of the phage lambda genome from the chromosome to re-establish virulent
229 infection. Indeed, several environmental stresses, such as UV light or chemicals (including
230 antibiotics), as well as inflammation in the GIT, can induce prophage excision (Banks et al.,
231 2003; Barnhart et al., 1976; Goerke et al., 2006). Oh et al. recently add dietary fructose and
232 short-chain fatty acids to the list of inducers of prophage excision, suggesting a mechanism
233 of phage-mediated alteration of the intestinal microbiota depending on the bacterial
234 metabolism (Oh et al., 2019). The induction of excision is essential for the perpetuation of
235 temperate phages, as it provides a means of infecting more bacteria and disseminating. The
236 exit of the phage from a bacterial chromosome must, therefore, be precisely controlled by a
237 defined molecular mechanism. This mechanism has been dissected in great detail for phage
238 lambda and is based on a genetic switch governing the production of the CI (lysogeny-
239 promoting) and Cro (excision/lytic-promoting) proteins. Most of the inducers of phage
240 excision provoke DNA damage, triggering an emergency response that is used by the phage
241 to express the genes required for the excision. However, signalling molecules also impact on
242 the decision for excision. A *Vibrio cholerae* phage encodes for a receptor able to activate the
243 phage lytic pathway upon binding to a quorum sensing molecule produced by the bacterial
244 host (Silpe and Bassler, 2018). An alternative system, called arbitrium, has recently been
245 described (Erez et al., 2017). Upon bacterial lysis, a peptide produced by the prophage is
246 released; when the concentration of this peptide in the environment exceeds a particular
247 threshold, the surrounding bacteria perceive the signal (through a set of genes also
248 originating from the phage), leading to the cessation of lysis and the promotion of lysogeny.
249 This novel system is thought to be only one of many original systems awaiting discovery
250 (Howard-Varona et al., 2017). In the confined intestinal environment, where accumulation of
251 small signalling molecules is favoured compared to open environments, such new
252 mechanisms are likely to play a role in shaping the microbial communities. Recent studies on

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

259

260 *Impact of phages on bacterial behaviour and virulence*

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

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

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

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

305

306 *Phages in disease cohorts*

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

314 One of the earliest studies of the richness and diversity of the phage community associated
315 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

348 study of complex microbial communities and the need to support phage therapy as one
349 possible solution to the problem of antibiotic resistance (Roach and Debarbieux, 2017).
350 When trying to understand what maintains or disturbs the intestinal microbial balance in
351 healthy subjects, efforts should focus on modelling and defining how different
352 coevolutionary models can concurrently shape microbial diversity in spatially heterogeneous
353 environments (Hannigan et al., 2018). The type and cost of resistance and counter-resistance
354 should be considered, together with the physiological advantages of colonising different
355 niches. The aim is to describe the coexistence of many different phages and bacterial hosts
356 in the microbiota mathematically, and to predict the sweeps likely to alter or maintain the
357 equilibrium in a steady state in the long term. Viral ecologists can provide a framework for
358 achieving this goal, generally by focusing on global ecosystems (Bolduc et al., 2017). The next
359 stage will be to develop models of these coevolution dynamics taking into account the
360 mammalian host in the context of both healthy and diseased subjects (Hochberg, 2018).
361 Experimental model systems will also be required to decipher individual mechanisms at the
362 molecular level. One-to-one interaction studies can reveal novel ways in which phages and
363 bacteria can manipulate each other's evolution, but access to the mammalian environment
364 remains limited. However, gnotobiotic murine models — germ-free mice colonised by a
365 defined set of bacterial strains —, are emerging as a surrogate system in which the impact of
366 the intestinal microbiota on health can be investigated. Germ-free mice colonised with
367 human bacterial strains have been used to describe phages from human viral faecal material
368 and could be further developed for the isolation of human-associated phages (Reyes et al.,
369 2013). Another model was recently established with murine bacterial strains, providing a
370 more natural intestinal ecosystem compared to introducing human bacterial strains into
371 germ-free mice. This model was then used to demonstrate the basis of the microbial
372 competition between *Salmonella typhimurium* and *E. coli* strains for the same niche
373 (Brugiroux et al., 2016). Such a model has the advantage of high reproducibility between
374 breeding facilities, making it possible for multiple teams with specific objectives to work with
375 the same tool. Murine models also provide biological samples that can be difficult to obtain
376 from humans, such as intestinal biopsy specimens, which are required to assess the
377 influence of spatial distribution on the interaction and evolution of microbial species. Last,
378 but not least, beyond PBI, diverse microbial interactions, such as those between fungi and

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

381 Finally, an area still overshadowed by the work on PBI and evolution is the effect of the
382 mammalian response to this dynamic consortium of microbes (Figure 3). Disease states, such
383 as inflammation in particular, have been shown to affect PBI, but many other processes
384 remain to be studied in the GIT and in human-associated microbiota in general (Diard et al.,
385 2017; Norman et al., 2015). Following on from the unexpected discovery that relationships
386 between microbiota and drugs can influence the efficacy of immunotherapy against tumors,
387 we can reasonably assume that some PBI play a role on biological processes beyond
388 microbiology (Routy et al., 2018). It remains unclear whether such effects are driven by the
389 antagonistic coevolution of phages and bacteria or a subtler diversion of function exploited
390 by the immune system, but these possibilities highlight how exciting phage biology has
391 become, as it enters a new era of full integration into studies of microbes and their
392 interactions within ecosystems.

393

394

395 Figure legends

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

405

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

416

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

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

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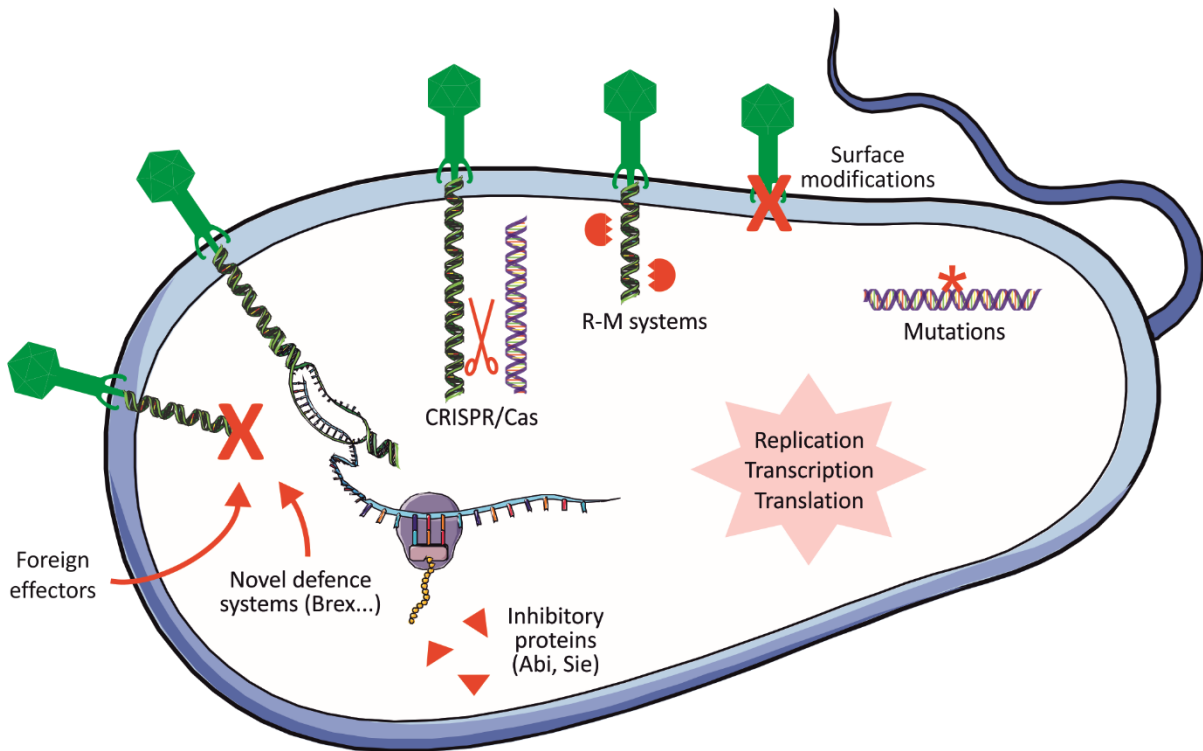
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654 Figure 1



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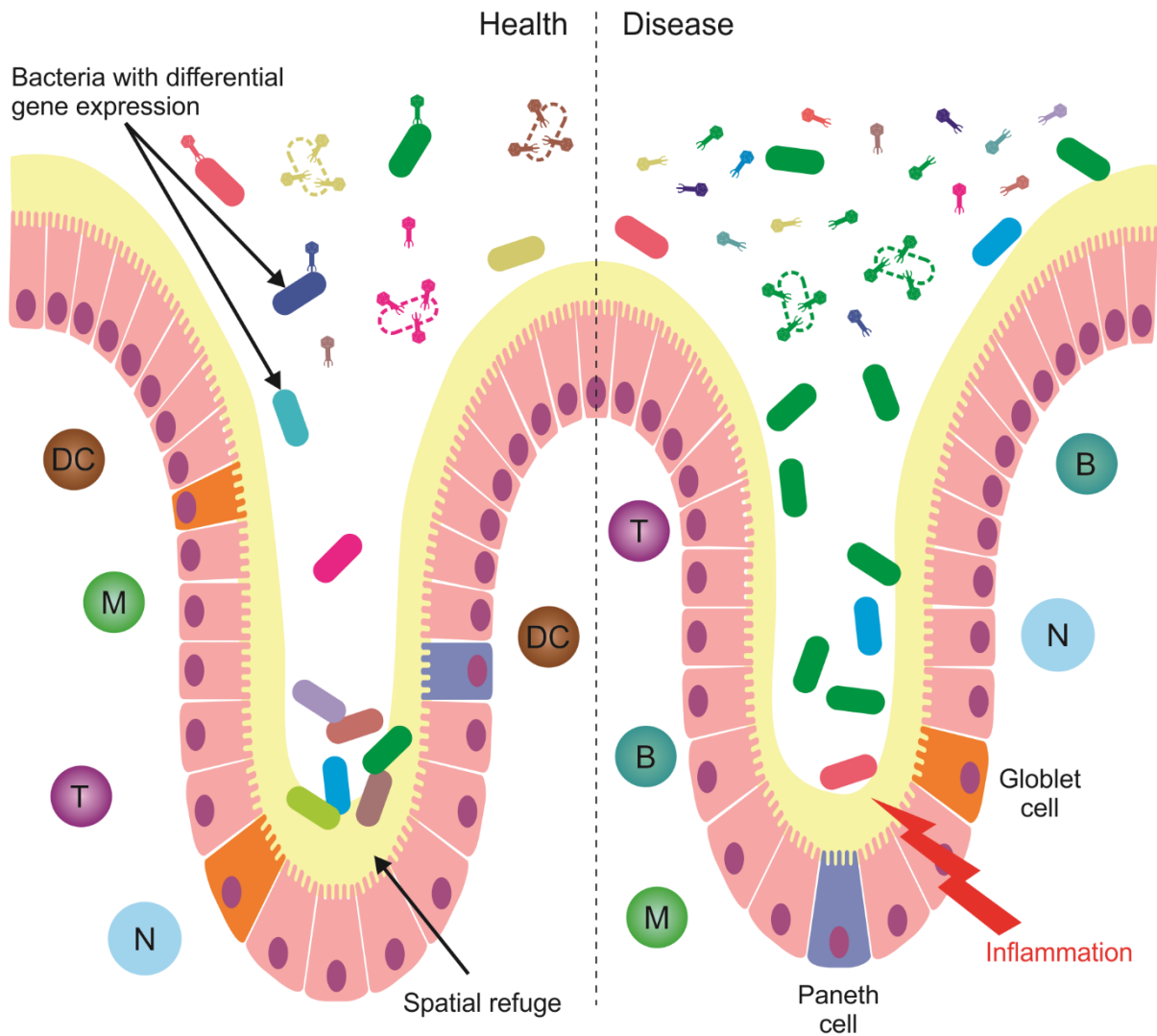
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657 Figure 1. Bacterial mechanisms of defence against phage predation. No single bacterium has
658 been found to possess all of these defence systems, but each bacterium can have several. A
659 large pink star indicates the essential mechanisms of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

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668 Figure 2

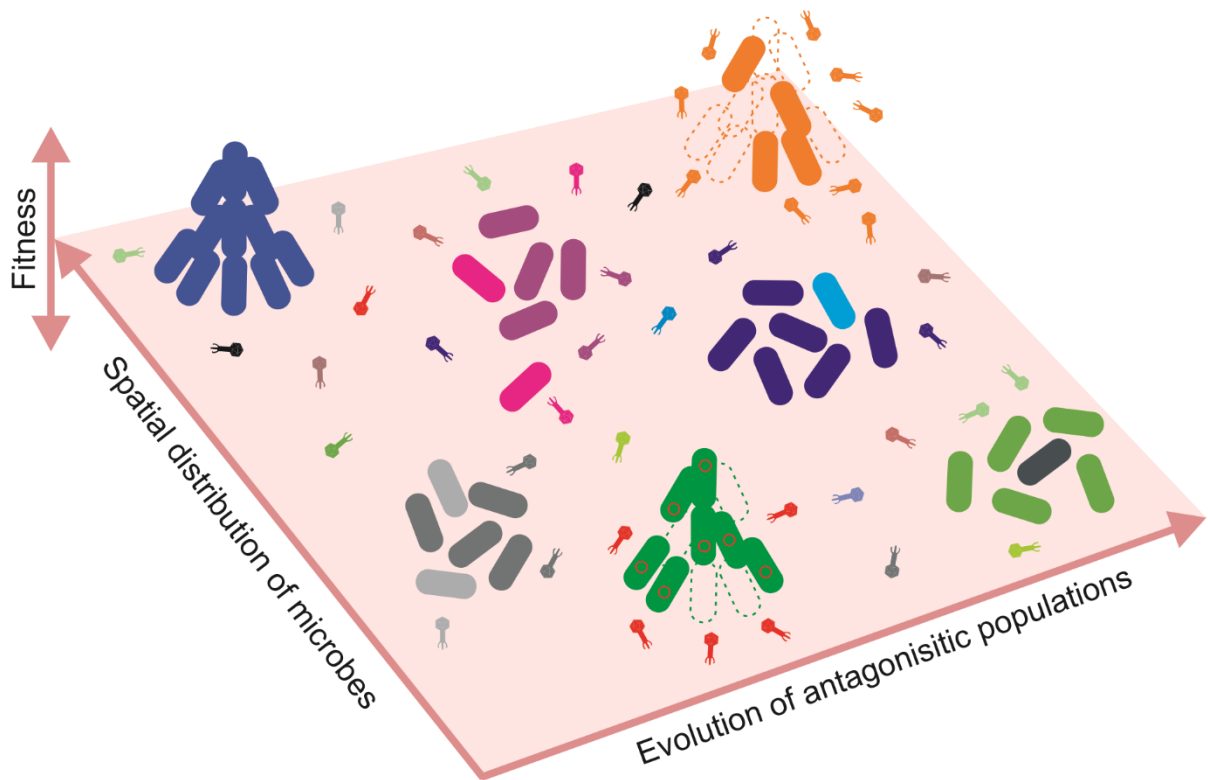


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676 phages with various colours). Intestinal villi can serve as spatial refuges for bacteria, enabling
677 them to escape phage predation, represented by dashed lines and multiple phages.
678 Epithelial cells and the mucus layer are coloured in rose and yellow, respectively, with the
679 rare goblet and Paneth cells noted. B, B cells; DC, dendritic cells; M, macrophages; N,
680 neutrophils; T, T cells.

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686 Figure 3. Schematic diagram of the antagonistic coevolution of phages and bacteria in a
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 693 resistant/inaccessible populations), orange (corresponding to bacteria lysed by virulent
 694 phages, dashed lines) or green (corresponding to lysogens from which prophage [red circles]
 695 excise). The mammalian host (represented as a pink area) underlies these microbial
 696 interactions and can modulate and be modulated by the outcome of these interactions.

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