Host-encoded DNA methyltransferases modify the epigenome and host tropism of invading phages

Michiko Takahashi[1¶*], Satoshi Hiraoka[2¶], Yuki Matsumoto[3], Rikako Shibagaki[1], Takako Ujihara[4], Hiromichi Maeda[1], Satoru Seo[1], Keizo Nagasaki[3], Hiroaki Takeuchi[5], Shigenobu Matsuzaki[6]

[1]Department of Surgery, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan
[2]Research Center for Bioscience and Nanoscience (CeBN), Research Institute for Marine Resources Utilization, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Kanagawa, Japan
[3]Faculty of Agriculture and Marine Science, Kochi University, Nankoku, Kochi, Japan
[4]Science Research Center, Kochi University, Nankoku, Kochi, Japan
[5]Department of Medical Laboratory Sciences, Health and Science, International University of Health and Welfare Graduate School, Narita, Chiba, Japan
[6]Faculty of Health Sciences, Kochi Gakuen University, Kochi, Kochi, Japan
[*]Correspondence: michikot@kochi-u.ac.jp
[¶]These authors contributed equally to this work.

SUMMARY

Restriction modification (RM) systems are ubiquitous bacterial defense systems; however, some phages evade RM system and adapt to their bacterial hosts. In such cases, phages are thought to stochastically acquire DNA methylation from host-encoded DNA methyltransferases (MTases), facilitating host adaptation. However, no studies have directly compared the methylomes of host bacteria and their infecting phages. Here, we demonstrate the epigenetic landscape of adapted phages with diverse infection histories, focusing on the broad host-range phage KHP30T as its adapts to three *Helicobacter pylori* strains. Using a multistage infection system, we observed that the adapted phages displayed significantly high titers against the last infected *H. pylori* strain, suggesting an attendant change in host tropism. Single-molecule real-time sequencing revealed that methylated motifs were predominantly shared between the adapted phages and their most recent host. Our findings enhance our understanding of epigenetic phage–host interactions, which have significant implications for microbial ecology.

KEYWORDS

Epigenome, methyltransferases, phage adaptation, phage-host interaction, phase variation, restriction-modification system, SMRT sequencing, *Helicobacter pylori*

INTRODUCTION

Epigenetic modifications regulate the activity of specific genes and cellular functions without altering DNA sequences. In eukaryotes, epigenetic modifications play a major role in various biological processes through modulating chromatin structure and organization.[1] In particular, DNA methylation, catalyzed by methyltransferases (MTases), is a key epigenetic signal discovered early in studies of DNA as genetic material.[2,3] In mammals, DNA methylation controls gene expression and chromatin structure, contributing to various cellular processes, including cell differentiation, genome imprinting, and X chromosome inactivation.[4]

As bacteria lack essential elements such as histones to structurally modify DNA, DNA methylation serves as a primary epigenetic regulator. [2] In prokaryotes, DNA methylation generally occurs in three forms: N6-methyladenine (m6A) and C5-methylcytosine (m5C), both also found in eukaryotes, and N4-methylcytosine (m4C), specific to bacteria and archaea.[2,5,6] Prokaryotic DNA methylation regulates multiple biological processes, including cell cycle, gene expression, virulence, and host defense.[6]

Historically, one of the most well-studied prokaryotic systems involved in DNA methylation is the restriction modification (RM) system that defends against foreign DNA such as conjugative plasmids and phages.[7–9] Most of these systems comprise an MTase and a restriction endonuclease (REase) that recognizes a specific target sequence for cleavage. These RM systems are classified into Types I, II, and III based on their subunit composition, cofactor requirements, recognition sequence structure, and mode of action.[10] REases interact with their target DNA sequences to cleave the DNA, whereas MTases methylate these recognition sites to protect them from cleavage.[10] Consequently, host genomic DNA is methylated by endogenous MTases and protected against REase digestion, whereas unmethylated foreign DNA is cleaved by REases. In contrast to these three major groups, Type IV systems consist solely of REase(s)

59 that recognize and cleave methylated DNA.[10,11]

60 Methods for detecting DNA methylation have been particularly well developed for
61 eukaryotic m5C, because the biological importance of m5C in mammalian cells over recent
62 decades.[12] However, these techniques are not directly applicable to bacterial methylomes, where
63 m6A and m4C are more prevalent. Several approaches, such as restriction enzyme assays, and
64 bisulfite sequencing, have been used to detect bacterial methylomes with higher prevalences of
65 m6A and m4C; however, these methods have some limitations: restriction enzyme assays cannot
66 detect unknown motifs, Sanger sequencing has low throughput, and bisulfite sequencing cannot
67 detect m6A and m4C.[13] Therefore, further development of DNA methylation detection methods
68 applicable to bacterial methylomes is needed.

69 Recent advances in sequencing technologies, including single-molecule real-time
70 (SMRT) sequencing have addressed this problem. SMRT sequencing was the first to enable
71 simultaneous detection of all three major forms of prokaryotic DNA methylation, leading to
72 extensive analysis of bacterial and archaeal methylomes and significant expansion of REBASE,
73 a database of information about components of RM systems.[13–18] In addition, methylome analysis
74 has been extended to viruses and phages.[19–22] Recent studies revealed that some DNA viruses
75 encode MTases in their genomes and use them as part of their replication strategy.[21,23,24] In
76 contrast, viruses that do not encode MTases can stochastically acquire DNA methylation from
77 host-encoded MTases during their adaptation to the host strain. Experiments with microbes
78 possessing well-characterized RM systems such as *Escherichia coli, Bacillus subtilis,*
79 *Haemophilus* spp., and *Salmonella* spp. suggest that phage genomes are modified by host-
80 encoded MTases during infection, allowing adapted phages to develop a “protective suit” against
81 the host RM systems.[25] However, no study has directly compared the methylomes of hosts and
82 phages to determine whether adapted phages acquire DNA methylation from host-encoded

83 MTases.

84 Here, we demonstrate the epigenomic landscape of phages with distrinct histories of
85 infection. To model bacteria-phage interactions mediated by host-encoded MTases, we
86 established a multistage infection system and generated nine adapted phages with different
87 infection histories using three strains of *Helicobacter pylori* and the *H. pylori* phage KHP30T. The
88 adapted phages showed high infectious titers against the strains of *H. pylori* infected in the last
89 generation but low titers against other strains. SMRT sequencing revealed that methylated motifs
90 in the KHP30T genome were shared with those in the last-infected *H. pylori* strains. These findings
91 suggest that the phage genome is modified by host-encoded MTases, resulting in the phage DNA
92 evading cleavage by cognate REases. Our results provide direct evidence that adapted phages
93 stochastically acquire DNA methylation from host bacteria and offer new insights relevant to
94 phage therapy design.

95

96 **RESULTS**

97 ***Titration of adapted phages***

98 We designed multistage infection systems using the KHP30T phage and three *H. pylori* strains
99 (26695, 3401T, and HPK5) to obtain adapted phages with different infection histories (Figures 1
100 and S1). Using this strategy, nine adapted phages (K2, K23, K232, K23H, K23H3, K2H, K2H2,
101 K2H3, and K2H3H) were retrieved. For example, K23 represents a KHP30T phage adapted to *H.*
102 *pylori* 26695 in the first stage and then to *H. pylori* 3401T in the second stage. Infectious titers
103 were measured using a plaque assay. Adapted phages showed high titers against the *H. pylori*
104 strain in the last generation of their adaptation but lower titers against non-adapted or previously
105 adapted hosts (Figures 2 and S2). For example, K2, which was adapted to *H. pylori* 26695 in the
106 first stage (Figure 1), showed a significantly higher titer (9.2 $\log_{10}$ plaque forming units [PFU]/mL)

against 26695, but a markedly lower titer against 3401T and HPK5 (3.1 and 3.9 $\log_{10}$ PFU/mL, respectively) ($p < 0.01$, Tukey–Kramer test). Focusing on second-stage phages, K23 showed a significantly higher titer (8.9 $\log_{10}$ PFU/mL) against *H. pylori* 3401T than against 26695 and HPK5 (0.8 and 1.8 $\log_{10}$ PFU/mL, respectively) ($p < 0.01$), even though K23 had adapted to 26695 in the first stage. K2H also showed the same trend: a titer against *H. pylori* HPK5 (8.2 $\log_{10}$ PFU/mL) that was significantly higher than that against 26695 and 3401T (1.9 and 2.4 $\log_{10}$ PFU/mL, respectively, $p < 0.01$) (Figure 2).

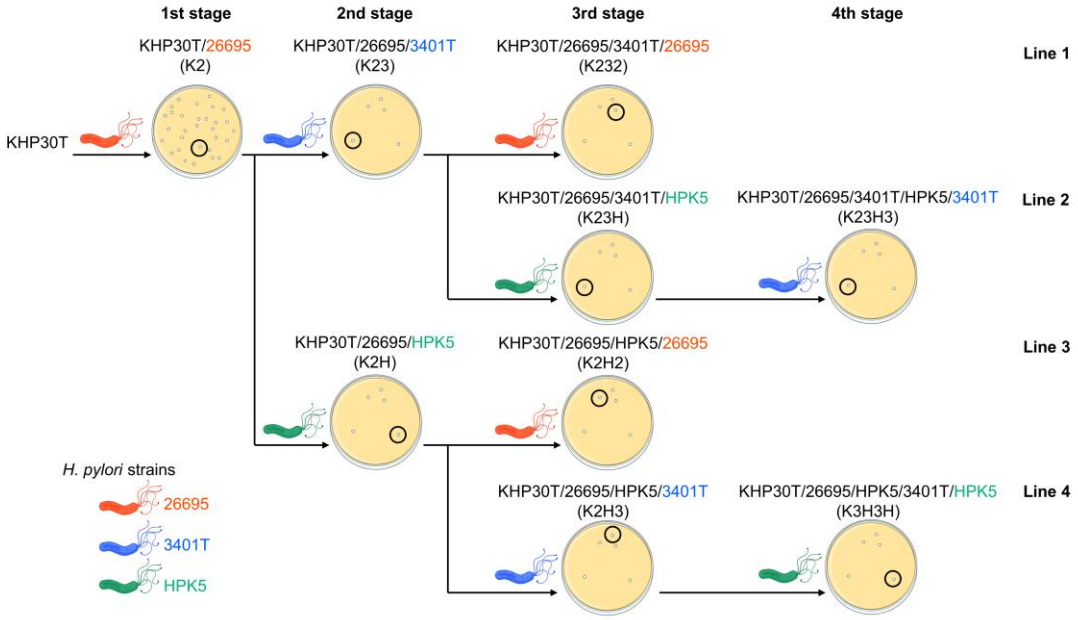

Figure 1. Overview of multistage infection system. Nine KHP30T phages were prepared by adapting to three strains of *H. pylori* (26695, 3401T, HPK5) through four infection stages and four lines. Details of the adaptation procedure are provided in Transparent Methods and Figure S1.

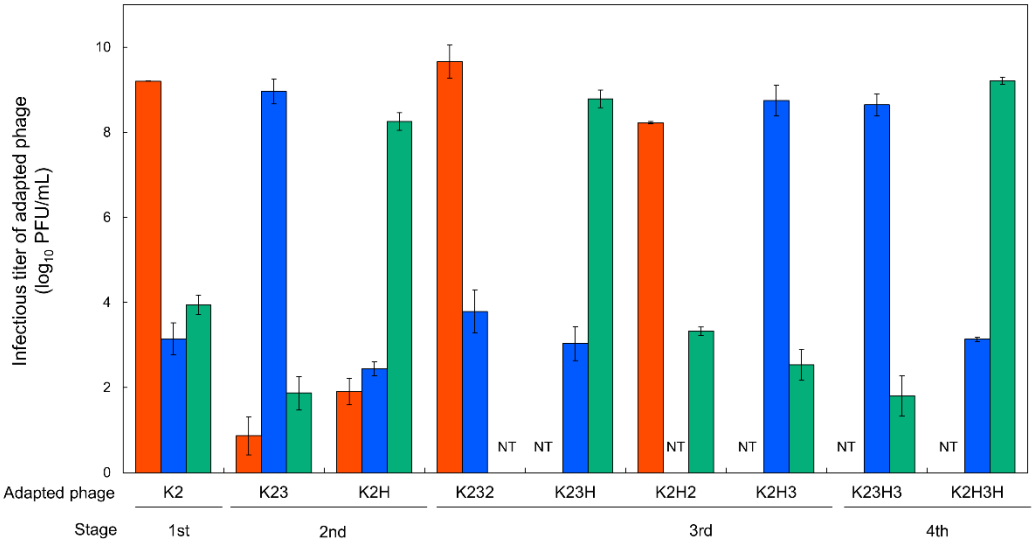

Figure 2. Infectious titer of adapted KHP30T. Orange, blue, and green bars represent KHP30T titers against *H. pylori* 26695, 3401T, and HPK5, respectively. Error bars represent standard deviation (n = 3).

Similarly, third-stage adapted phages showed titers against their most recent host strain that were higher than those against other strains. For example, K232 and K2H2 (lines 1 and 3 in Figure 1, respectively) showed significantly higher titers against *H. pylori* 26695 (9.6, and 8.2 $\log_{10}$ PFU/mL, respectively) than those against the other two strains ($p < 0.01$). A similar trend was observed in the fourth stage: K23H3 (line 2) showed a higher titer against 3401T (8.6 $\log_{10}$ PFU/mL) than that against HPK5 (1.8 $\log_{10}$ PFU/mL) ($p < 0.01$). K2H3H (line 4) showed a higher titer against *H. pylori* HPK5 (9.2 $\log_{10}$ PFU/mL) than that against 3401T (3.1 $\log_{10}$ PFU/mL, $p < 0.01$) (Figure 2).

When comparing between adapted phages, the infectious titers against each of the last host strains were generally similar. For example, K2, K232, and K2H2, which all shared *H. pylori* 26695 as their last host, showed titers >8.0 $\log_{10}$ PFU/mL against 26695 (range: 8.2–9.6 $\log_{10}$ PFU/mL) but significantly lower titers against other strains (range: 3.1–3.9 $\log_{10}$ PFU/mL).

135 Likewise, K23 and K23H3 showed significantly higher titers against 3401T (range: 8.5–8.6 $\log_{10}$
136 PFU/mL) than those against any other strain. Phages K2H, K23H, and K2H3H also showed
137 significantly higher titers against HPK5 (8.2–9.2 $\log_{10}$ PFU/mL) than those against the others
138 (Figure 2).

139 Additionally, we measured the infectious titers of the three adapted phages (K2, K23,
140 and K2H) after large-scale cultivation to evaluate whether host tropism changed with passaging.
141 The reproliferated phages exhibited the same trends as the original phages across all host
142 combinations; titers were highest against the most recent host but were lower against the other
143 hosts, including those previously adapted strains (Figure S3). Taken together, these results
144 suggest that the generation gap during reproliferation did not significantly affect the infectivity of
145 the adapted phages in this study.

146

147 ***Genomic analysis of host H. pylori strains and adapted phages***

148 SMRT sequencing of two *H. pylori* strains (3401T and HPK5) and three adapted phages (K2, K23,
149 and K2H) was performed to determine the epigenomes of both host strains and adapted phages
150 during infection. Genomic and epigenomic data for *H. pylori* 26695 were retrieved from the
151 National Center for Biotechnology Information (NCBI) RefSeq database (accession no.
152 CP003904.1) and a previously published report.[26] The complete circular genomes of *H. pylori*
153 3401T and HPK5 were obtained by de novo assembly using high fidelity (HiFi) reads (Table S1).
154 The general characteristics of these genomes (e.g., genome size, coding sequence [CDS]
155 number, and GC content) are similar to those of other *H. pylori* genomes deposited in the NCBI
156 database. The reconstructed *H. pylori* 3401T genome showed >99.99% average nucleotide
157 identity (ANI) with the ancestral strain *H. pylori* 3401 (AP024599.1).[27] Minor differences in the
158 sequences most likely arose from genomic mutations during serial subculturing and/or from

159 different sequencing technologies and bioinformatic software tools. No extrachromosomal content
160 was observed in any of the strains.

161 Using functional annotation with REBASE, we predicted 32–36 MTase and 16–21
162 REase genes in the three *H. pylori* genomes (Tables S1 and S2). The most abundant MTase type
163 was Type II, whereas Types I and III were nearly equivalent. A systematic search for phage
164 defense systems in the genomes revealed 14, 9, and 14 RM systems in *H. pylori* 26695, 3401T,
165 and HPK5, respectively (Table S3). More than half of the detected MTases were orphaned. The
166 most common modification was m6A, followed by m5C and m4C. Although it has been reported
167 that DNA methylation is also involved in mediating other defense systems (e.g., BREX[28] and
168 DISARM[29]), no other systems associated with DNA modification have been identified. In addition
169 to modification-mediated defense systems, six to nine other systems (e.g., AbiL[30] and
170 dynamins[31,32]) were identified in the three strains (Table S3).

171 Mapping KHP30T reads to the original KHP30 phage genome (AB647160.1)[33] identified
172 an approximately 2-kilobase (kb) insertion in all three phages. No single nucleotide
173 polymorphisms or short insertions/deletions (indels) were detected. The insertion matched a
174 segment of the *H. pylori* 3401 genome, indicating it may have been transferred from the host
175 during serial passaging. This insertion likely does not significantly affect the general
176 characteristics of the phage, such as infection efficiency or host tropism, consistent with the
177 titration results above (Figure 2) and previous reports.[33–35] The complete circular genome of
178 KHP30T was reconstructed using HiFi reads from the K2 phages (Table S1). Read mapping
179 analysis showed no obvious genomic variations fixed within the phage population. No MTase or
180 REase genes, as well as phage defense systems, were detected in the phage genome.

181

182 ***Epigenomic analysis of H. pylori strains and adapted phages***

183 We successfully identified numerous base modifications across both the *H. pylori* and phage
184 genomes (Figures 3 and S4, Table S4). From motif predictions, we detected 15 and 20 motifs in
185 the *H. pylori* 3401T and HPK5 genomes, respectively, as well as 17 motifs in *H. pylori* 26695, the
186 latter retrieved from a previous study[26] (Table S4). Among these motifs, the most abundant
187 modification type was m6A, followed by m4C and m5C. There was a slight discrepancy in the
188 modification types of the encoded MTases, possibly due to the SMRT sequencing's low detection
189 sensitivity for m5C modifications. As expected, the detected m5C motifs showed low modification
190 ratios (<50.0%) across all three *H. pylori* strains. Only two motifs, C**A**TG and G**A**TC (with the
191 modified bases in bold and their reverse complements underlined), were shared among all three
192 strains. However, 28 of 39 (72%) motifs were present in either of the 3 strains, indicating high
193 diversity in methylation patterns across these strains. This trend aligns with previous findings that
194 *H. pylori* strains possess highly divergent combinations of modified motifs.[26,36]

195 Next, we compared the methylation patterns in *H. pylori* strains and their adapted
196 phages. Overall, the methylated motifs were shared between pairs of *H. pylori* strains and their
197 respective adapated phages (Figure 3). For example, among the motifs detected in *H. pylori*
198 HPK5, with the exception of m5C motifs, 17 of 19 were highly modified in phage K2H (motif
199 modification ratios >68.9% and >63.6% in the host and phage genomes, respectively). Similarly,
200 12 of 13 motifs were shared between *H. pylori* 3401T and phage K23 with ratios >73.9% and
201 >41.7%, respectively. In addition, 12 of 14 motifs were concordantly methylated between *H. pylori*
202 26695 and phage K2, with ratios >95.2% and >71.3%, respectively. In contrast, sets of methylated
203 motifs were not shared between non-corresponding host-phage pairs.

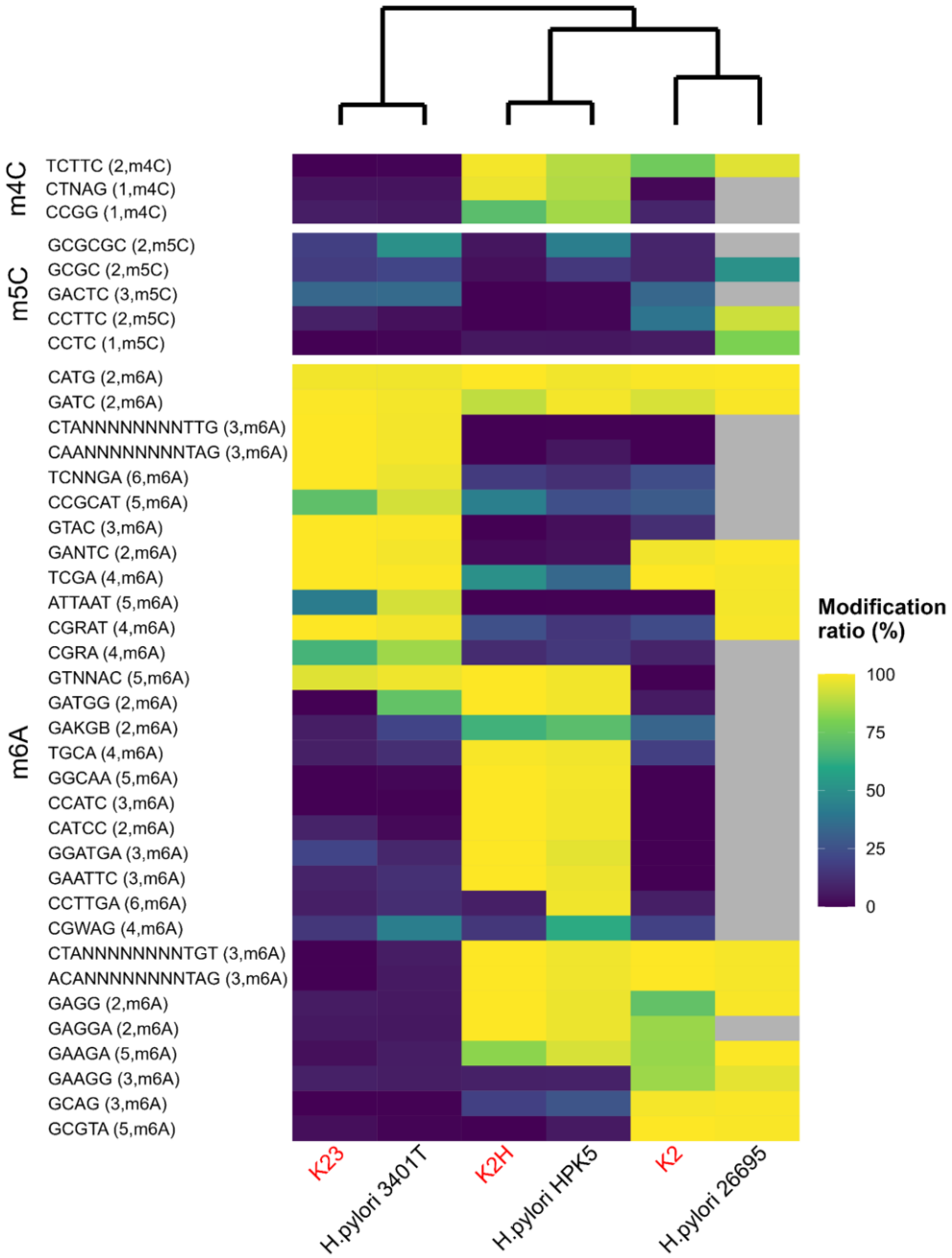

Figure 3. Methylomes of adapted phages and *H. pylori* strains. Methylated motifs were predicted from the three *H. pylori* strains. Motifs are shown separately according to methylation type. Phages and *H. pylori* strains are colored in red and black, respectively, in the bottom text. Text on the left side indicates the methylated motifs with methylated nucleotide position and methylation type. The top dendrogram was calculated using Euclidean distance and Ward method. For *H. pylori* 26695, methylated motifs and ratios were retrieved from the previous study reported by Krebes et al. (2014), and gray cells indicate motifs for which no data are available. Some motifs showed comparatively high modification ratios due to motif overlap. For example, the modification

ratio of CGR**A**T (R=A/G) in *H. pylori* 3401T is explained by motif overlapping with CGR**A**, which was detected from epigenomic analysis of the strain. In addition, CGW**A**G (W=A/T) in *H. pylori* 3401T partly overlapped with CGR**A**, i.e., CGA**A**G. A motif G**A**GG in *H. pylori* HPK5 also overlapped with the detected G**A**GGA from the strain, and in contrast, G**A**GGA in K2 overlap with G**A**GG reported from *H. pylori* 26695 previously. Among all seven CCGC**A**T sites in K23, two and one overlapped with other motifs C**A**TG (i.e., CCGC**A**TG) and **A**TCC (i.e., CCGC**A**TCC), respectively. Hence, CCGC**A**T showed a comparatively higher modification ratio in K23.

Notably, several motifs were identified that were highly methylated in the host genomes but showed minimal methylation in the adapted phage genomes (Figure 3). For instance, the m6A-type motif G**A**TGG was methylated in the *H. pylori* 3401T genome with a high modification ratio (73.9%), but was undetected in the K23 genome. Similarly, the motif CCTTG**A** was highly modified in *H. pylori* HPK5 (81.1%) but not in K2H (7.1%). Additionally, motifs A**T**TA**A**T and CGR**A**T have been reported to be methylated at high modification ratios (98.5% and 98.9%, respectively) in *H. pylori* 26695[26] but showed much lower modification in phage K2 (0.0% and 22.6%, respectively).

Phase-variable RM systems and associated genes

In the *H. pylori* HPK5 genome, a G9-tract was identified within the HPYHPK5_00170−00190 region (Figure S5A), displaying high sequence similarity to the M.Hpy300X Type III m6A MTase gene. This gene is known to induce methylome variation through simple sequence repeats (SSRs).[37] The basal state of the poly-G tract results in a stop codon and a truncated protein product. However, the addition of a single nucleotide to the tract results in a frameshift, generating a fully functional fusion protein. Similar to the Hpy300X system, this tract likely modulates MTase activity, suggesting that HPYHPK5_00150-00160 functions as a phase-variable Type III RM system in conjunction with a Type III REase (HPYHPK5_00170). Similar structures were also

239 predicted in *H. pylori* 26695, specifically in Type III RM systems AFV42588−42590 (HpyAXVII)
240 (Figure S5B) and AFV42742−42744 (Figure S5C), with the former previously characterized.[38]
241 In the *H. pylori* 3401T genome, HPY3401T_09270 and HPY3401T_09280 harbor the
242 C19- and C18-tracts, respectively (Figure S5D). These sequences showed the highest similarity
243 to the sequences of genes associated with the HpyAXVI system (89.8% and 89.0% identity to
244 HpyAXVI-mut2 and HpyAXVI-mut1, respectively). The HpyAXVI system, composed of Type II RM
245 genes (REase and MTase fusion genes), was originally identified in *H. pylori* 26695[26] (Figure S5E).
246 This system is inactive when a stop codon separates the two CDSs fillowing the first tract but
247 becomes active when a frameshift near the tract fuses the two CDSs. When active, the tract
248 produces a truncated protein that regulates MTase sequence specificity. Therefore,
249 HPY3401T_09270 and HPY3401T_09280 are likely phase-variable Type II RM systems. In
250 addition, HPYHPK5_13900 and HPYHPK5_13910 contain C14- and G15-tracts, respectively
251 (Figure S5F). The former showed the highest sequence similarity (88.2%) to Hpy300XI found in
252 *H. pylori* BCM-300 strain. This system uses two tracts to facilitate phase variation, likely operating
253 similarly to HpyAXVI[26]. Thus, HPYHPK5_13900 and HPYHPK5_13910 may also comprise
254 phase-variable Type II RM systems.
255 Beyond MTases, certain SSRs were associated with REase and specificity (S) subunit
256 genes. In all *H. pylori* genomes, four tracts were identified within Type I REase CDSs (Figure S5G,
257 S5H, S5I, and S5J). In contrast, cognate MTases and S genes were not associated with SSRs.
258 As Type I MTases require an S subunit complex for functionality, these tracts likely impact only
259 the cleavage activity of Type I RM systems, leaving methylation functions unaffected. In addition,
260 a C17-tract was found in the HPY3401T_11810 REase, part of a Type III RM system with the
261 HPY3401T_11820 MTase (Figure S5K). While Type III MTases function independently, REases
262 require a cognate MTase for cleavage. Tandem short-sequence repeats, including SSRs, are

263 common in Type III MTase genes and act as ON/OFF switches regulating phase variation,[39] as
264 also observed in this study (Figure S5A, S5B, and S5C). Therefore, this C17-tract may specifically
265 regulate only REase activity, while the cognate MTase may be constituitively active. AFV41850
266 from *H. pylori* 26695 and HPY3401T_02460 from 3401T showed the highest sequence similarity
267 to Type IV REase EcoCTGmrSD and possessed T15- and T19-tracts located 47 and 46 base
268 paris (bp) upstream of their CDSs, respectively (Figure S5L and S5M). Type IV REases cleave
269 methylated DNA strands in contrast to other REase types, suggesting that these tracts likely
270 regulate the ON/OFF switch for Type IV RM systems. Finally, consistent with a previous study's
271 findings, we confirmed that G14-tracts within the S gene comprise a BcgI-like Hpy99XXII system
272 accompanied by an upward-adjusted RM gene, though its function is still unclear[26] (Figure S5N).
273

274 **DISCUSSION**

275 DNA methylation-mediated biological processes affect a wide range of microbial ecologies,
276 including phage-host interactions. There is growing interest in epigenomic systems of diverse
277 prokaryotes and viruses, given their importance in microbial physiology, genetics, evolution, and
278 ecology.[2,40,41] To investigate the role of methylation in phage-host interactions, *H. pylori* and phage
279 KHP30 were chosen as model organisms.

280 *H. pylori* is a major human pathogen that causes chronic inflammation in the stomach
281 and gastric cancer. It harbors multiple RM systems with highly variable composition, resulting in
282 diverse methylomes across strains.[26,36,42–44] However, epigenomic evaluation has not been
283 performed to determine whether the MTases encoded in the *H. pylori* genome play a role against
284 invading phages. We previously reported that KHP30, a double-stranded DNA phage belonging
285 to the *Schmidvirus* genus, infects *H. pylori*[33–35] and has potential as a therapeutic agent against
286 antibiotic-resistant *H. pylori*, owing to its broad host range.[34] However, a significant barrier to

287 phage therapy for *H. pylori* lies in the limited understanding of its defense mechanisms against
288 KHP30 infection.

289 To clarify the relationship between DNA methylation and phage-host interactions, we
290 designed a multistage infection system (Figure 1) and analyzed the methylomes of three *H. pylori*
291 strains (26695, 3401T, and HPK5) along with three adapted KHP30T phages (K2, K23, and K2H)
292 with varying infection histories. The adapted phages showed high titers against their most recent
293 host strain but significantly lower titers against other strains, even if they had previously adapted
294 to them (Figure 2). Considering the short generation time during cultivation, gene expression
295 disruptions or enzymatic inactivation from mutations were unlikely to affect tropism significantly.
296 As expected, the reproliferated phages consistently showed high titers only against the latest
297 infected host (Figure S3).

298 Our epigenomic analysis revealed that each *H. pylori* strain contained distinct sets of
299 MTase genes, many positioned near REase genes within the RM systems (Tables S1 and S2).
300 Adapted phages were found to share methylated motifs with their final adapted host strain,
301 indicating that phage epigenomes were modified by host MTases during infection. Taken together,
302 our work demonstrates that adapted phages acquire DNA methylation from their host to evade
303 from the host's RM systems, thereby increasing infection efficiency, as has been previously
304 hypothesized.[45] To the best of our knowledge, this is the first study to directly observe epigenomic
305 changes in the phage genome during infection (Figure S4). Although methylated motifs were
306 shared between adapted phages and their host, exceptions were noted. Specifically, several
307 motifs were methylated in the host genome but not in the adapted phage (Figure 3). These
308 exceptions may partially be due to phase variations in several RM systems of *H. pylori* (Figure
309 S5).

310 Phase variation is a random and reversible switching mechanism in gene expression

311 that generates transcriptional, translational, and/or phenotypic diversity within bacterial
312 populations.[46] Bacterial MTases within RM systems often undergo phase variation, resulting in
313 heterogeneous cells with varied RM activity within a clonal population.[47] This heterogeneity
314 enables phages to selectively infect cells with reduced RM activity, as their epigenomes are
315 methylated by the MTases in these hosts. A primary mechanism regulating phase variation is the
316 presence of SSRs within or upstream of RM genes, with phase-variable ON/OFF switching
317 occuring in the former, resulting in reversible changes in the number of SSRs.[47–51] Our analysis
318 found several phase-variable regulons or phasevarions associated with RM genes in all three *H.*
319 *pylori* genomes (Figure S5). Further single-cell evaluation such as combining
320 immunofluorescence imaging to track phase-variated proteins[52] with fluorescence *in situ*
321 hybridization for phage detection, are necessary to clarify the link between phase variation and
322 phage variation. Moreover, bioinformatic tools for detecting modifications needs to be improved,
323 as current modification detection tools do not account for phase-variable epigenomes and instead
324 treat modifications on a binary, population-wide manner. Another possible explanation for the
325 methylated motifs found exclusively in the host genome is the potential presence of anti-RM
326 systems in the phage that block host MTase activity. For example, DarA and DarB in P1 phages
327 protect recognition sites on their genome from host RM activity[53,54]. Similarly, Ocr in T7 phages
328 mimics DNA structure to block the DNA-binding groove of Type I REases/MTases.[55] However, no
329 genes associated with known anti-RM systems[56] were found in the KHP30T genome.

330 Defense systems are involved in the evolutionary arms race between phages and
331 hosts.[57] Epigenomic modifications significantly influence their co-evolution and promote microbial
332 diversification.[58,59] Our findings revealed that the phage epigenome was altered by host bacteria
333 during infection, enhancing the phage's ability to reinfect the same host strain (Figures 2 and 3).
334 To counter the epigenomic synchronization of invading phages and avoid extinction of the entire

335 population by phage persistence, some bacteria employ variable phage-defense systems. For
336 example, many species of gut bacteria, such as *Bacteroides fragilis* and *H. pylori*, show strain-
337 specific variations in gene sets associated with phage defense, including MTase genes.[60–62] While
338 gene acquisition or deletion rely on genomic events such as horizontal gene transfer and
339 recombination, such events occur at infrequently. Alternatively, a more dynamic strategy by
340 bacteria involves phase variation to prevent phage circulation. For example, *Vibrio cholerae*
341 modulates the expression of the lipopolysaccharide O1 antigen through phase variation to escape
342 predation by O1-specific phages.[63] In *H. pylori*, inter-strain variation in methylomes caused by
343 MTase phase variation has been reported.[36,37] Therefore, antagonistic coevolution between
344 prokaryotes and phages, along with associated epigenetic changes, is of great interest for
345 understanding the diversity within microbial ecosystems.

346 In summary, our study demonstrates that phages can overcome host RM systems by
347 acquiring methylation modifications from host-encoded MTases during infection. Adapted phages
348 showed significantly higher titers against their most recent host strain, likely due to neutralization
349 of the host RM system through acquired methylation. Our results enhance our understanding of
350 DNA methylation-mediated phage–host interactions and their impact on microbial ecology across
351 diverse environments.

352 Our approach provides new insights into the design of therapeutic phage cocktails.
353 Phage therapy holds promise across various fields, including human and plant diseases,[64–67]
354 owing to phages' advantages such as host specificity, self-amplification, biofilm degradation, and
355 low toxicity to humans.[68,69] One of the primary challengess in phage therapy is bacterial
356 resistance; however, phage cocktails, as shown in studies on *E. coli* O157:H7 and *Klebsiella*
357 *pneumoniae*, offer a strategy to combat resistance by incorporating diverse phage variants.[70,71]
358 We propose that a cocktail of adapted phages, each with distinct methylation motifs, could

359 improve the chances of overcoming host modification-mediated defense systems, such as RM
360 systems. Further research to understand the mechanism of phage adaptation and evaluate their
361 therapeutic potential will contribute to the development of tailored phage cocktails for medical and
362 industrial applications.
363
364 ***Limitation of the study***
365 This study has few limitations. First, we did not experimentally verify the activity and specificity of
366 the MTases and REases identified, leaving uncertainty about which RM systems are actively
367 involved in phage defense and the specific MTases responsible for the modified motifs. Second,
368 although SMRT sequencing technology efficiently identifies m6A and m4C modifications,[26] it has
369 limited sensitivity for detecting m5C modifications, one of the most common methylation types in
370 nature after m6A and m4C. In total, five m5C motifs were detected across all three *H. pylori* strains
371 (Table S4), but they showed low modification ratios (Figure 3, Table S4), presumably due to the
372 low detection accuracy for this modification type using current SMRT technology. In addition to
373 methylation, other epigenetic modifications, such as phosphorothioate modification, have recently
374 been linked to phage defense.[72] Expanding sequencing technologies to detect a wider range of
375 DNA modifications is crucial for a comprehensive understanding of epigonomic functions. Third,
376 although HiFi reads are highly accurate (>99.9%), sequencing of homopolymer regions remains
377 challenging,[73–75] complicating estimatations of tract diversity. Nonetheless, given the high genome
378 coverage achieved in this study (Table S1), the tracts associated with RM genes (Figure S5) likely
379 represent the predominant phase variation state in the populations studied.
380
381 **RESOURCE AVAILABILITY**
382 ***Lead contact***
383 Further information and requests for resources and reagents should be directed to and will be

fulfilled by the lead contact, Michiko Takahashi (michikot@kochi-u.ac.jp).

Data availability

The assembled genomes were deposited in DDBJ/ENA/GenBank (S1 Table). All data are registered under BioProject ID PRJDB17787 [https://ddbj.nig.ac.jp/resource/bioproject/PRJDB17787]. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHODS

All methods can be found in the Transparent Methods.

ACKNOWLEDGMENTS

The authors are greatful to Waka Ishida, Yusaku Funaoka, and Takahiro Ishikawa for experimental support, and to Daichi Morimoto for helpful comments on this work. This study was supported by JSPS KAKENHI (grant numbers JP20K15582 and JP22K08875), ACT-X, Japan Science and Technology Agency (grant numbers JPMJAX21BD and JPMJAX22BK), and the budget of Kochi University Hospital (Japan). The experiments were conducted at the Division of Biological Research, Science Research Center, Kochi University. Computations were partially performed on the supercomputer system at the Institute for Chemical Research, Kyoto University, and the NIG supercomputer system at ROIS National Institute of Genetics, and the Data Analysis and the Earth Simulator systems at JAMSTEC.

AUTHOR CONTRIBUTIONS

MT designed the study, acquired funding, performed the experiments, and drafted the manuscript. SH designed the study, performed the bioinformatics analyses, and wrote the manuscript. YM, RS, and TU performed the experiments. HM acquired funding and provided resources. SS, KN, and HT supervised the project. SM conceived the original idea, acquired funding, and supervised

the project. All authors have read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest associated with this manuscript.

SUPPLEMENTAL INFORMATION

Figure S1. Overview of preparation procedure for adapted phages.

Figure S2. Tukey-Kramer pairwise comparisons of phage titers. Corresponding titers are shown in Figure 2. Combination of phage and host strain are indicated as 'adapted phage': 'host strain used for titration' format in the x-axis: for example, 'K2:2' means a titer of K2 against *H. pylori* 26695. Solid black bar within each box represents median. Upper alphabet summarizes the results of the Tukey-Kramer test; letters denote significant differences ($p < 0.01$) in mean titers with groups a > b > c > d > e > f.

Figure S3. Infectious titer of reproliferated phages. (A) Orange, blue, and green bars represent titer of reproliferated KHP30T phages to *H. pylori* 26695, 3401T, and HPK5, respectively. Error bars indicate standard deviation ($n = 3$). (B) Tukey-Kramer pairwise comparisons of reproliferated phage titers. Phage and host combinations are indicated in the same format as Figure S2. Infectious titer of the 'Original phages' is derived from Figure 2. Solid black bar within each box represents median. Upper alphabet summarizes the results of the Tukey-Kramer test; letters denote significant differences ($p < 0.01$) in mean titers with groups a > b > c > d > e > f.

Figure S4. Methylomes of adapted phages. Circos plot showing the distribution of DNA methylation. Orange, blue, and red bars represent m4C, m5C, and m6A modifications, respectively. K2 and K23 were sequenced using PacBio Sequel II in CCS mode, whereas K2H was sequenced using PacBio Sequel in CLR mode.

Figure S5. Genetic organization of RM system genes associated with SSRs. Homopolymeric tracts inside or upstream of RM genes were specified as SSRs. RM gene types are indicated by color and text inside to each CDS. CDSs not assigned to RM system genes are colored in gray.

Table S1. Descriptions and statistics of *H. pylori* strains and KHP30T phages.

Table S2. Detected RM system genes in *H. pylori* strains.

Table S3. Predicted phage defense systems in *H. pylori* strains.

Table S4. Methylated motifs predicted from *H. pylori* strains.

Transparent Methods

443 **REFERENCES**

444 1. Liyanage, V., Jarmasz, J., Murugeshan, N., Del Bigio, M., Rastegar, M., and Davie, J. (2014).
445 DNA modifications: Function and applications in normal and disease states. Biology *3*, 670–
446 723.
447 2. Sánchez-Romero, M.A., and Casadesús, J. (2020). The bacterial epigenome. Nat. Rev.
448 Microbiol. *18*, 7–20.
449 3. Avery, O.T., Macleod, C.M., and McCarty, M. (1944). Studies on the chemical nature of the
450 substance inducing transformation of pneumococcal types : Induction of transformation by a
451 desoxyribonucleic acid fraction isolated from Pneumococcus Type III. J. Exp. Med. *79*, 137–
452 158.
453 4. Razin, A., and Riggs, A.D. (1980). DNA methylation and gene function. Science *210*, 604–
454 610.
455 5. Weigele, P., and Raleigh, E.A. (2016). Biosynthesis and function of modified bases in
456 bacteria and their viruses. Chem. Rev. *116*, 12655–12687.
457 6. Seong, H.J., Han, S.-W., and Sul, W.J. (2021). Prokaryotic DNA methylation and its functional
458 roles. J. Microbiol. *59*, 242–248.
459 7. Labrie, S.J., Samson, J.E., and Moineau, S. (2010). Bacteriophage resistance mechanisms.
460 Nat. Rev. Microbiol. *8*, 317–327.
461 8. Ando, T., Ishiguro, K., Watanabe, O., Miyake, N., Kato, T., Hibi, S., Mimura, S., Nakamura,
462 M., Miyahara, R., Ohmiya, N., et al. (2010). Restriction-modification systems may be
463 associated with *Helicobacter pylori* virulence. J. Gastroenterol. Hepatol. *25*, S95-8.
464 9. Arber, W. (1974). DNA modification and restriction. Prog. Nucleic Acid Res. Mol. Biol. *14*, 1–
465 37.
466 10. Ershova, A.S., Rusinov, I.S., Spirin, S.A., Karyagina, A.S., and Alexeevski, A.V. (2015). Role
467 of restriction-modification systems in prokaryotic evolution and ecology. Biochemistry *80*,
468 1373–1386.
469 11. Mruk, I., and Kobayashi, I. (2014). To be or not to be: regulation of restriction-modification
470 systems and other toxin-antitoxin systems. Nucleic Acids Res. *42*, 70–86.
471 12. Plongthongkum, N., Diep, D.H., and Zhang, K. (2014). Advances in the profiling of DNA
472 modifications: cytosine methylation and beyond. Nat. Rev. Genet. *15*, 647–661.
473 13. Beaulaurier, J., Schadt, E.E., and Fang, G. (2019). Deciphering bacterial epigenomes using
474 modern sequencing technologies. Nat. Rev. Genet. *20*, 157–172.
475 14. Roberts, R.J., Vincze, T., Posfai, J., and Macelis, D. (2015). REBASE--a database for DNA
476 restriction and modification: enzymes, genes and genomes. Nucleic Acids Res. *43*, D298-9.
477 15. Forde, B.M., McAllister, L.J., Paton, J.C., Paton, A.W., and Beatson, S.A. (2019). SMRT
478 sequencing reveals differential patterns of methylation in two O111:H- STEC isolates from a

hemolytic uremic syndrome outbreak in Australia. Sci. Rep. *9*, 9436.

16. Murray, I.A., Clark, T.A., Morgan, R.D., Boitano, M., Anton, B.P., Luong, K., Fomenkov, A., Turner, S.W., Korlach, J., and Roberts, R.J. (2012). The methylomes of six bacteria. Nucleic Acids Res. *40*, 11450–11462.
17. Hiraoka, S., Okazaki, Y., Anda, M., Toyoda, A., Nakano, S.-I., and Iwasaki, W. (2019). Metaepigenomic analysis reveals the unexplored diversity of DNA methylation in an environmental prokaryotic community. Nat. Commun. *10*, 159.
18. Roberts, R.J., Vincze, T., Posfai, J., and Macelis, D. (2023). REBASE: a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res. *51*, D629–D630.
19. Coy, S.R., Gann, E.R., Papoulis, S.E., Holder, M.E., Ajami, N.J., Petrosino, J.F., Zinser, E.R., Van Etten, J.L., and Wilhelm, S.W. (2020). SMRT sequencing of Paramecium bursaria chlorella virus-1 reveals diverse methylation stability in adenines targeted by restriction modification systems. Front. Microbiol. *11*, 887.
20. Sun, C., Chen, J., Jin, M., Zhao, X., Li, Y., Dong, Y., Gao, N., Liu, Z., Bork, P., Zhao, X.-M., et al. (2023). Long-read sequencing reveals extensive DNA methylations in human gut phagenome contributed by prevalently phage-encoded methyltransferases. Adv. Sci. *10*, e2302159.
21. Jeudy, S., Rigou, S., Alempic, J.-M., Claverie, J.-M., Abergel, C., and Legendre, M. (2020). The DNA methylation landscape of giant viruses. Nat. Commun. *11*, 2657.
22. Hiraoka, S., Sumida, T., Hirai, M., Toyoda, A., Kawagucci, S., Yokokawa, T., and Nunoura, T. (2022). Diverse DNA modification in marine prokaryotic and viral communities. Nucleic Acids Res. *50*, 1531–1550.
23. Hoelzer, K., Shackelton, L.A., and Parrish, C.R. (2008). Presence and role of cytosine methylation in DNA viruses of animals. Nucleic Acids Res. *36*, 2825–2837.
24. Wilson, W.H., Van Etten, J.L., and Allen, M.J. (2009). The Phycodnaviridae: the story of how tiny giants rule the world. Curr. Top. Microbiol. Immunol. *328*, 1–42.
25. Krüger, D.H., and Bickle, T.A. (1983). Bacteriophage survival: multiple mechanisms for avoiding the deoxyribonucleic acid restriction systems of their hosts. Microbiol. Rev. *47*, 345–360.
26. Krebes, J., Morgan, R.D., Bunk, B., Spröer, C., Luong, K., Parusel, R., Anton, B.P., König, C., Josenhans, C., Overmann, J., et al. (2014). The complex methylome of the human gastric pathogen *Helicobacter pylori*. Nucleic Acids Res. *42*, 2415–2432.
27. Takahashi, M., Matsumoto, Y., Ujihara, T., Maeda, H., Hanazaki, K., Nagasaki, K., Takeuchi, H., and Matsuzaki, S. (2021). Complete genome sequence of *Helicobacter pylori* strain 3401, a suitable host for bacteriophages KHP30 and KHP40. Microbiol Resour Announc *10*,

515 e0064721.
516 28. Goldfarb, T., Sberro, H., Weinstock, E., Cohen, O., Doron, S., Charpak-Amikam, Y., Afik, S.,
517 Ofir, G., and Sorek, R. (2015). BREX is a novel phage resistance system widespread in
518 microbial genomes. EMBO J. *34*, 169–183.
519 29. Ofir, G., Melamed, S., Sberro, H., Mukamel, Z., Silverman, S., Yaakov, G., Doron, S., and
520 Sorek, R. (2017). DISARM is a widespread bacterial defence system with broad anti-phage
521 activities. Nat. Microbiol. *3*, 90–98.
522 30. Deng, Y.-M., Liu, C.-Q., and W. Dunn, N. (1999). Genetic organization and functional analysis
523 of a novel phage abortive infection system, AbiL, from *Lactococcus lactis*. J. Biotechnol. *67*,
524 135–149.
525 31. Millman, A., Melamed, S., Leavitt, A., Doron, S., Bernheim, A., Hör, J., Garb, J., Bechon, N.,
526 Brandis, A., Lopatina, A., et al. (2022). An expanded arsenal of immune systems that protect
527 bacteria from phages. Cell Host Microbe *30*, 1556-1569.e5.
528 32. Guo, L., Sattler, L., Shafqat, S., Graumann, P.L., and Bramkamp, M. (2022). A bacterial
529 dynamin-like protein confers a novel phage resistance strategy on the population level in
530 *Bacillus subtilis*. MBio *13*. https://doi.org/10.1128/mbio.03753-21.
531 33. Uchiyama, J., Takeuchi, H., Kato, S.-I., Takemura-Uchiyama, I., Ujihara, T., Daibata, M., and
532 Matsuzaki, S. (2012). Complete genome sequences of two *Helicobacter pylori*
533 bacteriophages isolated from Japanese patients. J. Virol. *86*, 11400–11401.
534 34. Uchiyama, J., Takeuchi, H., Kato, S.-I., Gamoh, K., Takemura-Uchiyama, I., Ujihara, T.,
535 Daibata, M., and Matsuzaki, S. (2013). Characterization of *Helicobacter pylori* bacteriophage
536 KHP30. Appl. Environ. Microbiol. *79*, 3176–3184.
537 35. Kamiya, R., Uchiyama, J., Matsuzaki, S., Murata, K., Iwasaki, K., and Miyazaki, N. (2022).
538 Acid-stable capsid structure of *Helicobacter pylori* bacteriophage KHP30 by single-particle
539 cryoelectron microscopy. Structure *30*, 300-312.e3.
540 36. Furuta, Y., Namba-Fukuyo, H., Shibata, T.F., Nishiyama, T., Shigenobu, S., Suzuki, Y.,
541 Sugano, S., Hasebe, M., and Kobayashi, I. (2014). Methylome diversification through
542 changes in DNA methyltransferase sequence specificity. PLoS Genet. *10*, e1004272.
543 37. Nell, S., Estibariz, I., Krebes, J., Bunk, B., Graham, D.Y., Overmann, J., Song, Y., Spröer, C.,
544 Yang, I., Wex, T., et al. (2018). Genome and methylome variation in *Helicobacter pylori* with
545 a *cag* pathogenicity island during early stages of human infection. Gastroenterology *154*,
546 612-623.e7.
547 38. Prasad, Y., Kumar, R., Chaudhary, A.K., Dhanaraju, R., Majumdar, S., and Rao, D.N. (2019).
548 Kinetic and catalytic properties of M.HpyAXVII, a phase-variable DNA methyltransferase
549 from *Helicobacter pylori*. J. Biol. Chem. *294*, 1019–1034.
550 39. Rao, D.N., Dryden, D.T.F., and Bheemanaik, S. (2014). Type III restriction-modification

enzymes: a historical perspective. Nucleic Acids Res. *42*, 45–55.

40. Srikhanta, Y.N., Fox, K.L., and Jennings, M.P. (2010). The phasevarion: phase variation of type III DNA methyltransferases controls coordinated switching in multiple genes. Nat. Rev. Microbiol. *8*, 196–206.
41. Furuta, Y., and Kobayashi, I. (2012). Mobility of DNA sequence recognition domains in DNA methyltransferases suggests epigenetics-driven adaptive evolution. Mob. Genet. Elements *2*, 292–296.
42. Ando, T., Xu, Q., Torres, M., Kusugami, K., Israel, D.A., and Blaser, M.J. (2000). Restriction-modification system differences in *Helicobacter pylori* are a barrier to interstrain plasmid transfer. Mol. Microbiol. *37*, 1052–1065.
43. Alm, R.A., Ling, L.S., Moir, D.T., King, B.L., Brown, E.D., Doig, P.C., Smith, D.R., Noonan, B., Guild, B.C., deJonge, B.L., et al. (1999). Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature *397*, 176–180.
44. Bubendorfer, S., Krebes, J., Yang, I., Hage, E., Schulz, T.F., Bahlawane, C., Didelot, X., and Suerbaum, S. (2016). Genome-wide analysis of chromosomal import patterns after natural transformation of *Helicobacter pylori*. Nat. Commun. *7*, 11995.
45. Isaev, A.B., Musharova, O.S., and Severinov, K.V. (2021). Microbial arsenal of antiviral defenses - Part I. Biochemistry *86*, 319–337.
46. de Vries, N., Duinsbergen, D., Kuipers, E.J., Pot, R.G.J., Wiesenekker, P., Penn, C.W., van Vliet, A.H.M., Vandenbroucke-Grauls, C.M.J.E., and Kusters, J.G. (2002). Transcriptional phase variation of a type III restriction-modification system in *Helicobacter pylori*. J. Bacteriol. *184*, 6615–6623.
47. Atack, J.M., Tan, A., Bakaletz, L.O., Jennings, M.P., and Seib, K.L. (2018). Phasevarions of bacterial pathogens: methylomics sheds new light on old enemies. Trends Microbiol. *26*, 715–726.
48. Gor, V., Ohniwa, R.L., and Morikawa, K. (2021). No change, no life? What we know about phase variation in *Staphylococcus aureus*. Microorganisms *9*, 244.
49. Nahar, N., Tram, G., Jen, F.E.-C., Phillips, Z.N., Weinert, L.A., Bossé, J.T., Jabbari, J.S., Gouil, Q., Du, M.R.M., Ritchie, M.E., et al. (2023). *Actinobacillus pleuropneumoniae* encodes multiple phase-variable DNA methyltransferases that control distinct phasevarions. Nucleic Acids Res. *51*, 3240–3260.
50. Moxon, R., Bayliss, C., and Hood, D. (2006). Bacterial contingency loci: the role of simple sequence DNA repeats in bacterial adaptation. Annu. Rev. Genet. *40*, 307–333.
51. Bayliss, C.D. (2009). Determinants of phase variation rate and the fitness implications of differing rates for bacterial pathogens and commensals. FEMS Microbiol. Rev. *33*, 504–520.
52. Ben-Assa, N., Coyne, M.J., Fomenkov, A., Livny, J., Robins, W.P., Muniesa, M., Carey, V.,

587 Carasso, S., Gefen, T., Jofre, J., et al. (2020). Analysis of a phase-variable restriction
588 modification system of the human gut symbiont *Bacteroides fragilis*. Nucleic Acids Res. *48*,
589 11040–11053.
590 53. Iida, S., Streiff, M.B., Bickle, T.A., and Arber, W. (1987). Two DNA antirestriction systems of
591 bacteriophage P1, *darA*, and *darB*: characterization of *darA*- phages. Virology *157*, 156–166.
592 54. Teklemariam, A.D., Al-Hindi, R.R., Qadri, I., Alharbi, M.G., Ramadan, W.S., Ayubu, J., Al-
593 Hejin, A.M., Hakim, R.F., Hakim, F.F., Hakim, R.F., et al. (2023). The battle between bacteria
594 and bacteriophages: a conundrum to their immune system. Antibiotics *12*, 381.
595 55. Roberts, G.A., Stephanou, A.S., Kanwar, N., Dawson, A., Cooper, L.P., Chen, K., Nutley, M.,
596 Cooper, A., Blakely, G.W., and Dryden, D.T.F. (2012). Exploring the DNA mimicry of the Ocr
597 protein of phage T7. Nucleic Acids Res. *40*, 8129–8143.
598 56. Samson, J.E., Magadán, A.H., Sabri, M., and Moineau, S. (2013). Revenge of the phages:
599 defeating bacterial defences. Nat. Rev. Microbiol. *11*, 675–687.
600 57. Hampton, H.G., Watson, B.N.J., and Fineran, P.C. (2020). The arms race between bacteria
601 and their phage foes. Nature *577*, 327–336.
602 58. Scanlan, P.D. (2017). Bacteria-bacteriophage coevolution in the human gut: implications for
603 microbial diversity and functionality. Trends Microbiol. *25*, 614–623.
604 59. Dimitriu, T., Szczelkun, M.D., and Westra, E.R. (2020). Evolutionary ecology and interplay of
605 prokaryotic innate and adaptive immune systems. Curr. Biol. *30*, R1189–R1202.
606 60. O'Connell Motherway, M., Zomer, A., Leahy, S.C., Reunanen, J., Bottacini, F., Claesson, M.J.,
607 O'Brien, F., Flynn, K., Casey, P.G., Munoz, J.A.M., et al. (2011). Functional genome analysis
608 of *Bifidobacterium breve* UCC2003 reveals type IVb tight adherence (Tad) pili as an essential
609 and conserved host-colonization factor. Proc. Natl. Acad. Sci. U. S. A. *108*, 11217–11222.
610 61. Zhu, A., Sunagawa, S., Mende, D.R., and Bork, P. (2015). Inter-individual differences in the
611 gene content of human gut bacterial species. Genome Biol. *16*, 82.
612 62. Yawata, Y., Kiyokawa, T., Kawamura, Y., Hirayama, T., Takabe, K., and Nomura, N. (2019).
613 Intra- and interspecies variability of single-cell innate fluorescence signature of microbial cell.
614 Appl. Environ. Microbiol. *85*, e00608-19.
615 63. Seed, K.D., Faruque, S.M., Mekalanos, J.J., Calderwood, S.B., Qadri, F., and Camilli, A.
616 (2012). Phase variable O antigen biosynthetic genes control expression of the major
617 protective antigen and bacteriophage receptor in *Vibrio cholerae* O1. PLoS Pathog. *8*,
618 e1002917.
619 64. Pires Diana P., Cleto Sara, Sillankorva Sanna, Azeredo Joana, and Lu Timothy K. (2016).
620 Genetically engineered phages: a review of advances over the last decade. Microbiol. Mol.
621 Biol. Rev. *80*, 523–543.
622 65. Abedon, S.T., Kuhl, S.J., Blasdel, B.G., and Kutter, E.M. (2011). Phage treatment of human

623 infections. Bacteriophage *1*, 66–85.
624 66. Balogh, B., Jones, J.B., Iriarte, F.B., and Momol, M.T. (2010). Phage therapy for plant disease
625 control. Curr. Pharm. Biotechnol. *11*, 48–57.
626 67. Loc Carrillo, C., Atterbury, R.J., el-Shibiny, A., Connerton, P.L., Dillon, E., Scott, A., and
627 Connerton, I.F. (2005). Bacteriophage therapy to reduce *Campylobacter jejuni* colonization
628 of broiler chickens. Appl. Environ. Microbiol. *71*, 6554–6563.
629 68. Lin, D.M., Koskella, B., and Lin, H.C. (2017). Phage therapy: an alternative to antibiotics in
630 the age of multi-drug resistance. World J. Gastrointest. Pharmacol. Ther. *8*, 162–173.
631 69. Loc-Carrillo, C., and Abedon, S.T. (2011). Pros and cons of phage therapy. Bacteriophage *1*,
632 111–114.
633 70. Tanji, Y., Shimada, T., Yoichi, M., Miyanaga, K., Hori, K., and Unno, H. (2004). Toward rational
634 control of *Escherichia coli* O157:H7 by a phage cocktail. Appl. Microbiol. Biotechnol. *64*, 270–
635 274.
636 71. Gu, J., Liu, X., Li, Y., Han, W., Lei, L., Yang, Y., Zhao, H., Gao, Y., Song, J., Lu, R., et al.
637 (2012). A method for generation phage cocktail with great therapeutic potential. PLoS One
638 *7*, e31698.
639 72. Jiang, S., Chen, K., Wang, Y., Zhang, Y., Tang, Y., Huang, W., Xiong, X., Chen, S., Chen, C.,
640 and Wang, L. (2023). A DNA phosphorothioation-based Dnd defense system provides
641 resistance against various phages and is compatible with the Ssp defense system. MBio *14*,
642 e0093323.
643 73. Wenger, A.M., Peluso, P., Rowell, W.J., Chang, P.-C., Hall, R.J., Concepcion, G.T., Ebler, J.,
644 Fungtammasan, A., Kolesnikov, A., Olson, N.D., et al. (2019). Accurate circular consensus
645 long-read sequencing improves variant detection and assembly of a human genome. Nat.
646 Biotechnol. *37*, 1155–1162.
647 74. Mc Cartney, A.M., Shafin, K., Alonge, M., Bzikadze, A.V., Formenti, G., Fungtammasan, A.,
648 Howe, K., Jain, C., Koren, S., Logsdon, G.A., et al. (2022). Chasing perfection: validation
649 and polishing strategies for telomere-to-telomere genome assemblies. Nat. Methods *19*,
650 687–695.
651 75. Hebert, P.D.N., Braukmann, T.W.A., Prosser, S.W.J., Ratnasingham, S., deWaard, J.R.,
652 Ivanova, N.V., Janzen, D.H., Hallwachs, W., Naik, S., Sones, J.E., et al. (2018). A Sequel to
653 Sanger: amplicon sequencing that scales. BMC Genomics *19*, 219.