ANTIBIOTIC RESISTANCE

Invertible promoters mediate bacterial phase variation, antibiotic resistance, and host adaptation in the gut

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Phase variation, the reversible alternation between genetic states, enables infection by pathogens and colonization by commensals. However, the diversity of phase variation remains underexplored. We developed the PhaseFinder algorithm to quantify DNA inversion–mediated phase variation. A systematic search of 54,875 bacterial genomes identified 4686 intergenic invertible DNA regions (invertons), revealing an enrichment in host-associated bacteria. Invertons containing promoters often regulate extracellular products, underscoring the importance of surface diversity for gut colonization. We found invertons containing promoters regulating antibiotic resistance genes that shift to the ON orientation after antibiotic treatment in human metagenomic data and in vitro, thereby mitigating the cost of antibiotic resistance. We observed that the orientations of some invertons diverge after fecal microbiota transplant, potentially as a result of individual-specific selective forces.

Phase variation is a process that bacteria use to generate frequent and reversible changes within specific hypermutable loci, introducing phenotypic diversity into clonal populations. Such phenotypic diversity plays an important role in mediating preemptive adaptation to abrupt and severe selective events and is often crucial for infection by pathogens and colonization by commensals (*1*–*5*). In bacteria, phase variation often manifests through regions of DNA that invert between two states in a predictable, reversible manner (*6*). The mechanism of inversion involves enzymes called invertases, which recognize a set of inverted repeats flanking the invertible DNA region and catalyze its inversion in a reversible manner (*7*). Invertible regions commonly contain promoters oriented such that in the ON orientation, the promoter is poised to activate transcription of an operon (*7*). In the opposite OFF orientation, the promoter is oriented away from the operon, which is therefore not transcribed (*7*). Additional types of regulatory elements, such as terminators, may also be contained within these invertible DNA regions (*8*). Invertases catalyze frequent inversions—for example, one inversion in every 100 to 1000 *Escherichia coli* cells, a rate at least three orders of magnitude higher than the rate of point mutations (*7*, *9*, *10*). Thus, invertible promoters generate genetic diversity in populations, enabling rapid and reversible adaptation. Studies in specific pathogens and commensals have reported invertible promoters that regulate genes involved in virulence or colonization, such as those that encode fimbriae, flagella, and capsular polysaccharides (*1*, *2*, *4*, *7*, *11*–*17*).

Phase variation mediated by DNA inversion is an underexplored mechanism with broad consequences for adaptation to abrupt and severe selective events. Here, we sought to systematically identify invertons, which we define as single intergenic invertible DNA regions flanked by inverted repeats likely recognized and inverted by invertase proteins in a reversible manner. The term inverton encompasses invertible promoters and intergenic invertible DNA regions containing alternate types of regulatory regions. Through our systematic search for invertons, we aimed to address three long-standing questions regarding this mechanism of regulation: (i) How prevalent are invertons? (ii) What are the functions of genes regulated by invertons? (iii) In the context of a host, do individual-specific selective pressures modulate inverton orientation? We found that invertons are widely distributed across bacteria, yielding fundamental insights into bacterial infection and colonization. We confirmed and expanded upon previous observations that the orientations of some invertons regulating capsular polysaccharide biosynthesis operons of human gut bacteria are stable within individuals and divergent between individuals (*17*). Using a fecal microbiota transplant (FMT) study to observe the orientation of invertons from the same strain in multiple individuals, we observed divergences in orientation between donor and patient. We also identified invertible promoters regulating antibiotic resistance genes. We observed that antibiotic treatment results in a shift from the OFF to ON orientation of these invertons in humans, and confirmed that antibiotics cause the orientation shift in vitro, which could mitigate the fitness cost of maintaining antibiotic resistance genes in the absence of antibiotics.

We developed the PhaseFinder algorithm to computationally identify invertons and quantify their orientations with genomic or metagenomic sequencing reads by identifying regions flanked by inverted repeats, mimicking their inversion in silico, and identifying regions where sequencing reads support both orientations (figs. S1 and S2). Simulations to benchmark the performance of PhaseFinder reveal that given enough coverage, PhaseFinder can identify most invertons without a substantial rate of false positives. We reasoned that if inversion rates were high, both orientations of invertons would coexist, allowing for the identification of invertons in bacterial populations used for genome sequencing. Therefore, we used PhaseFinder to search for invertons in all available NCBI genomes from RefSeq that were sequenced using Illumina paired-end sequencing with data deposited in the NCBI Short Read Archive. In total, 54,875 bacterial genomes spanning the breadth of cultured bacterial diversity were searched, leading to the discovery of 4686 putative invertons in 2414 genomes (tables S1 and S2). Invertons were found in 10 of 19 bacterial phyla. Five phyla harbored invertons in at least 20% of their genomes (Table 1). The lack of a systematic method to identify invertons was the impetus for our study; however, the limited scope of known inverton examples may lead to biases in the PhaseFinder algorithm against invertons with features divergent from known invertible promoters. Additionally, the identification of invertons with PhaseFinder relies on the presence of both orientations of the inverton in sequenced samples. Therefore, applying the PhaseFinder algorithm to additional bacterial genomes derived from diverse conditions and sequenced at higher coverage or with longer reads will likely lead to the discovery of many more invertons.

To explore how invertons are distributed across environmental niches, we used information from ProGenomes and the Joint Genome Institute to categorize species into aquatic, terrestrial, and host-associated habitats (*18*, *19*). The prevalence of invertons was higher in host-associated species [Fisher exact test, host versus aquatic FDR (false discovery rate) $P = 3.5 \times 10^{-5}$, odds ratio = 6.4; host versus terrestrial FDR $P = 0.0053$, odds ratio = 4.8) (Fig. 1A and table S3). This overall enrichment in the prevalence of invertons is due to phylum-level enrichment in Bacteroidetes (FDR $P = 2.35 \times 10^{-15}$) and Proteobacteria (FDR $P = 8.51 \times 10^{-5}$) and the fact that all Spirochaetes and Verrucomicrobia found with invertons were associated with vertebrate hosts. Additionally, we observed an increase in the number of invertons per genome

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in host-associated species (Wilcoxon rank sum test, host versus aquatic FDR $P = 0.00023$, W = 361,190; host versus terrestrial FDR $P = 0.007$, W = 180,680) (Fig. 1B). The enrichment of invertons in host-associated species is not due to habitat-specific differences in coverage (fig. S3). Overall, our results suggest that diverse species likely use invertons to increase their fitness in host-associated niches.

We acquired detailed information on the niches inhabited by species from the phylum Bacteroidetes (table S4). In Bacteroidetes, the prevalence of invertons was higher in host gut–associated species (Fisher exact test, host-gut versus aquatic FDR $P = 3.3 \times 10^{-35}$, odds ratio = 328.4; host-gut versus terrestrial FDR $P = 2.3 \times 10^{-34}$, odds ratio = 220.8; host-gut versus host-other, FDR $P = 3.9 \times 10^{-17}$, odds ratio = 35.2) (Fig. 1C). The number of invertons per genome was also higher in host gut–associated isolates (Wilcoxon rank sum test, host-gut versus aquatic FDR $P = 1.8 \times 10^{-26}$, W = 6911; host-gut versus terrestrial FDR $P = 2.3 \times 10^{-26}$, W = 6967; host-gut versus host-other FDR $P = 4.9 \times 10^{-13}$, W = 3956) (Fig. 1D).

Because of the observed enrichment for invertons in gut species, we performed an in-depth analysis and curation of invertons in a nonredundant selection of 49 representative species from human stool using longitudinal metagenomic data instead of the reads used to assemble reference genomes (table S5). We identified 459 putative invertons (table S6), 87.6% of which were from species in the phylum Bacteroidetes, which had an average of 19 invertons per genome. We also identified invertons in additional phyla. We found 53 invertons from two *Akkermansia* species (phylum Verrucomicrobia), two invertons from a *Eubacterium* species (phylum Firmicutes), and one inverton from a *Bifidobacterium* species (phylum Actinobacteria) (fig. S4).

We categorized the invertons according to their flanking inverted repeats (IR) and identified four canonical motifs in Bacteroidetes: three corresponding to known IR motifs in *B. fragilis* and one uncharacterized motif (Fig. 2A and tables S7 and S8) (*14*). We also identified a distinctive motif class with tandem repeats within each

Table 1. Invertons per phylum identified in a systematic search of bacterial genomes with PhaseFinder.

Phylum	Genomes searched	Genomes with invertons	Percentage of genomes with invertons	Total invertons
Acidobacteria	6	0	0	0
Actinobacteria	5,262	67	1.3	200
Armatimonadetes	2	0	0	0
Bacteroidetes	491	160	32.6	1,254
Chlamydiae	45	3	6.7	5
Chloroflexi	1	0	0	0
Cyanobacteria	20	4	20	7
Deinococcus-Thermus	14	1	7.1	1
Fibrobacteres	16	0	0	0
Firmicutes	17,010	986	5.8	1,422
Fusobacteria	10	0	0	0
Nitrospirae	1	0	0	0
Proteobacteria	14,872	1,133	7.6	1,628
Spirochaetes	138	57	41.3	140
Synergistetes	8	2	25	2
Tenericutes	21	0	0	0
Thermodesulfobacteria	3	0	0	0
Thermotogae	7	0	0	0
Verrucomicrobia	5	1	20	27

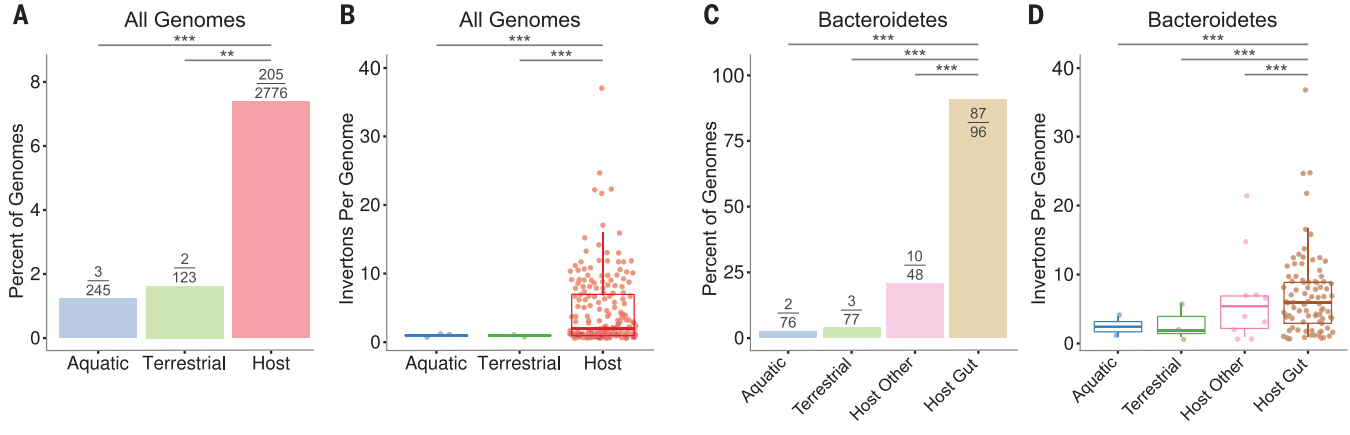

Fig. 1. Prevalence and number of invertons per genome are enriched in host-associated species. (**A** and **B**) The percentage of genomes identified with invertons (A) and the number of invertons per genome (B) from aquatic, terrestrial, and host-associated isolates. (**C** and **D**) In the phylum Bacteroidetes, the percentage of genomes identified with invertons (C) and the number of invertons per genome (D) from aquatic, terrestrial, host sites other than gut, and host gut–associated isolates. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

inverted repeat, which we call motif 0 (fig. S5). We found conserved promoter consensus motifs in 98% (231/235) of invertons with IR motifs 1 to 4 (Fig. 2B) (*20*). In contrast, promoter motifs were not observed in any of the invertible regions of IR motif 0–containing sequences (table S9). Because of the lack of promoter motifs combined with their location downstream of operons, invertons containing motif 0 may function as another type of regulatory element, such as a phase-variable terminator (*8*). In gut Bacteroidetes, we identified a total of 255 invertible promoters. On the basis of the orientation of the promoter consensus motif in relation to surrounding genes, we determined which genes or operons were regulated by invertible promoters and whether the promoter was in the ON or OFF orientation with respect to the downstream gene (tables S7 and S8). In both species of *Akkermansia*, all invertons are flanked by inverted repeats with the same motif (Fig. 2C), contain a promoter motif (Fig. 2D), and lack upstream invertases, which suggests that they are all invertible promoters co-regulated by a single, master invertase (EAJ16_05345 in *Akkermansia muciniphila*; MK095134 in *Akkermansia* sp. aa_0143).

Through functional annotation, we found that 73% (228/312) of invertible promoters regulate genes involved in the biosynthesis of polysaccharides, fimbriae, outer membrane proteins, and autotransporters; genes involved in the utilization of polysaccharides; or genes encoding PEP-CTERM domain–containing proteins (Fig. 2A, tables S7 and S8, and fig. S4). All of these functional categories except polysaccharide utilization are enriched in genes regulated by invertible promoters (tables S10 and S11). Genes regulated by invertible promoters are enriched for cell surface products (e.g., GO:0016020, membrane, $P = 1.93 \times 10^{-14}$) (table S11). The most enriched functional class is capsular polysaccharide biosynthesis loci, for which we found at least one example regulated by an inverton in four of the five major gut phyla: Bacteroidetes, Actinobacteria, Verrucomicrobia, and Firmicutes (table S11). Previous studies show that a repertoire of phase-variable capsular polysaccharides is necessary for competitive gut colonization by *Bacteroides* species (*2*, *16*, *21*). Our data show that phase-variable capsular polysaccharide biosynthesis loci are not just a peculiarity of *Bacteroides* species but are likely a convergent response to a strong selective mechanism in the vertebrate gut that possibly originates from the immune response or phages (*2*, *21*).

We found that invertible promoters could regulate antibiotic resistance genes, such as IBP132, which is upstream of the macrolide resistance gene *ermG* in *B. stercoris*. To investigate this mechanism in vivo, we searched specifically for antibiotic resistance genes regulated by invertible promoters in a cohort of 39 Finnish children, 19 of whom had never been exposed to antibiotics and 20 of whom had been administered 9 to 15 antibiotic treatments over a 3-year period (*22*). By coupling PhaseFinder with metagenomic assembly analysis, we found three antibiotic resistance genes regulated by invertible promoters: (i) the same *ermG* macrolide resistance gene as IBP132, (ii) a *cmeABC* multidrug resistance cassette conferring resistance primarily to macrolides and cephalosporins, and (iii) *pmrEL* genes conferring resistance to cationic antimicrobial peptides such as polymixin B (Fig. 3A). At least one antibiotic resistance gene regulated by an invertible promoter was found in 38% (15/39) of individuals from this Finnish cohort: 40% (8/20) of individuals who were administered antibiotics and 37% (7/19) of individuals who were untreated. Invertons regulating antibiotic resistance genes were also detected in metagenomic data from healthy adults in the United States. All examples of invertons regulating antibiotic resistance were found in *Bacteroides* species, which are increasingly associated with multi–drug-resistant infections (*23*). Surprisingly, all *cmeABC* and *ermG* antibiotic resistance genes were regulated by an identical invertible promoter. On the basis of genomic context, the *cmeABC/ermG* invertible promoter is likely located on an integrative conjugative element homologous to CTNhyb, an antibiotic resistance–transmitting mobile element (fig. S6) (*24*).

We examined the orientation of the invertible promoters regulating antibiotic resistance genes in longitudinal metagenomic data from the Finnish children. The mean orientation of the *cmeABC/ermG* invertible promoter was 94% OFF in untreated individuals and 84% OFF in individuals administered antibiotics. We observed an individual in which the *cmeABC/ermG* invertible promoter was 99% OFF 7 days before the macrolide azithromycin was administered and 74% ON 27 days after treatment (Fig. 3B). The *cmeABC/ermG* invertible promoter reverted to 99% OFF within 5 months after azithromycin administration (Fig. 3B). A similar phenomenon was observed in a second individual (fig. S7). A permutation test revealed that macrolide treatment was positively associated with the ON orientation of the *cmeABC/ermG* invertible promoter [quantitative polymerase chain reaction (qPCR) $P = 0.0005$, metagenomic $P = 0.0465$]. Thus, it appears that macrolides may select for the ON orientation of the *cmeABC/ermG* invertible promoter, and the orientation of the invertible promoter drifts toward OFF after cessation of antibiotic treatment.

To test whether antibiotics select for resistance genes with invertible promoters in the ON orientation, we first verified that the genes regulated by the *cmeABC/ermG* invertible promoter confer macrolide resistance. We cultivated 13 *B. stercoris* isolates with and one isolate without the *cmeABC/ermG* invertible promoter upstream of the macrolide resistance gene *ermG* (table S12) (*25*). The invertible promoter was primarily ON (>75%) in 10 isolates derived from erythromycin-containing media and primarily OFF (>97%) in three isolates derived from media

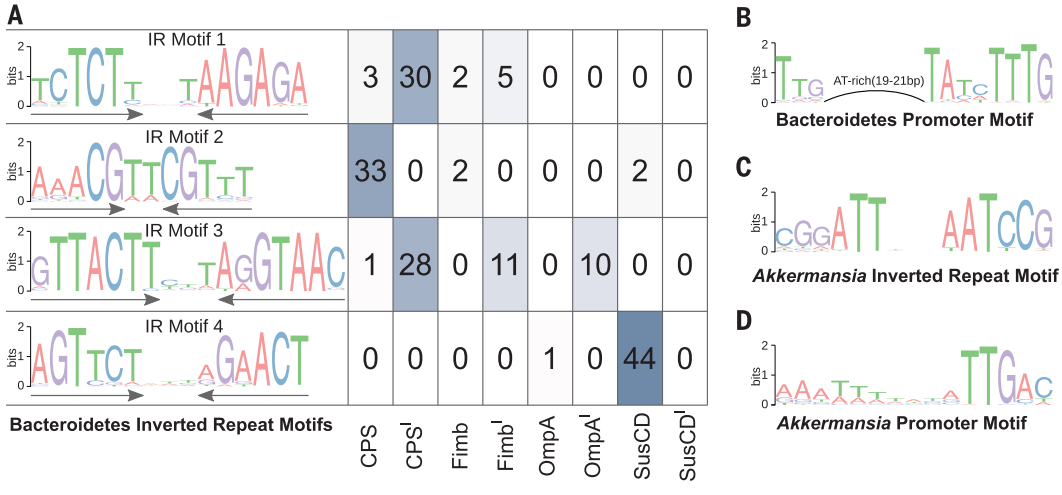

Fig. 2. Motifs found in the inverted repeats of Bacteroidetes and *Akkermansia* invertons consist of five to seven base pair palindromes with two or three intervening base pairs. (**A**) Functional profiling of operons regulated by invertons reveals specializations of each inverted repeat (IR) motif. The heat map represents the number of operons per functional class regulated by invertons with either global or local invertases. CPS, capsular polysaccharide; Fimb, fimbriae; OmpA, outer membrane protein A; SusCD, starch utilization system proteins C and D. Superscript I indicates the presence of a local invertase. The absence of local invertases directly upstream suggests that IR motifs 2 and 4 are likely regulated by global invertases. (**B**) Promoter motif identified from invertons from Bacteroidetes species. (**C**) The inverted repeat motif found in all identified invertons from *Akkermansia* spp. (**D**) Promoter motif identified from invertons from *Akkermansia* spp.

without erythromycin (Fig. 3C). We established that all *B. stercoris* isolates with *ermG* regulated by the *cmeABC/ermG* invertible promoter were resistant to erythromycin, whereas the isolate without the *ermG* gene was susceptible (fig. S8).

Next, we showed that erythromycin treatment selects for the ON orientation of the *cmeABC/ermG* invertible promoter. To quantify changes in the relative abundances of cells with the *cmeABC/ermG* invertible promoter in ON and OFF orientations, we performed qPCR comparing relative amplification using a static primer downstream of the invertible promoter paired with either a primer that amplifies the ON orientation or one that amplifies the OFF orientation (fig. S9). We transferred *B. stercoris* isolates with the *cmeABC/ermG* invertible promoter

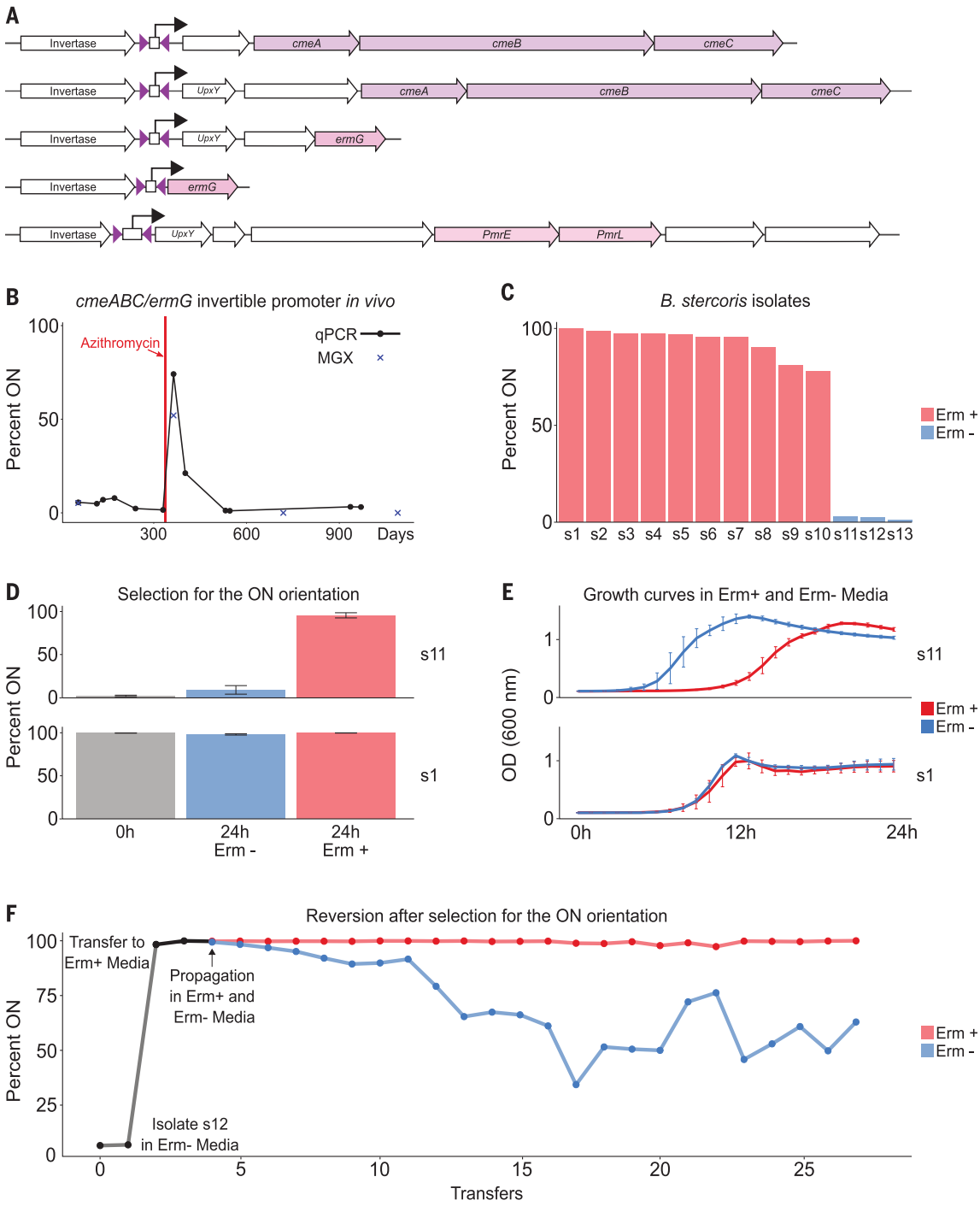

Fig. 3. Invertible promoters regulate antibiotic resistance genes. (**A**) Three classes of genes conferring antibiotic resistance are regulated by invertible promoters. The genomic contexts of the loci are shown with antibiotic resistance genes colored. Promoters are designated by hooked arrows; purple triangles represent inverted repeats. (**B**) An invertible promoter regulating the *cmeABC* multidrug efflux cassette in individual E011878 was 99% OFF before antibiotic treatment, shifted to 74% ON 7 days afterward, and then drifted back to 99% OFF over 5 months as measured by both qPCR and metagenomic (MGX) data. (**C**) The *cmeABC/ermG* invertible promoter is oriented ON in *B. stercoris* isolated in medium containing erythromycin (Erm+) and OFF in *B. stercoris* isolated in medium without erythromycin (Erm–). (**D**) The orientation of the *cmeABC/ermG* invertible promoter under antibiotic selection. Isolate s11 grown in Erm– medium remained OFF, whereas the same isolate transferred to Erm+ medium shifted to ON. Isolate s1 remained ON in Erm– and Erm+ media. (**E**) Growth of isolates under antibiotic selection. Isolate s1 grew in Erm+ medium without a lag phase relative to Erm– medium, whereas isolate s11 grew in Erm+ medium after an extended lag phase, consistent with a lower number of initially erythromycin-resistant cells. (**F**) Kinetics of the *cmeABC/ermG* invertible promoter. An isolate with the *cmeABC/ermG* invertible promoter initially in the OFF orientation, s12, was exposed to erythromycin. Half of the culture was then propagated in the presence of erythromycin and the other half in the absence of erythromycin for 24 1:1000 dilution transfers. In Erm+ medium, the promoter remained ON, whereas in Erm– medium, it drifted toward OFF. Error bars in (D) and (E) denote SD.

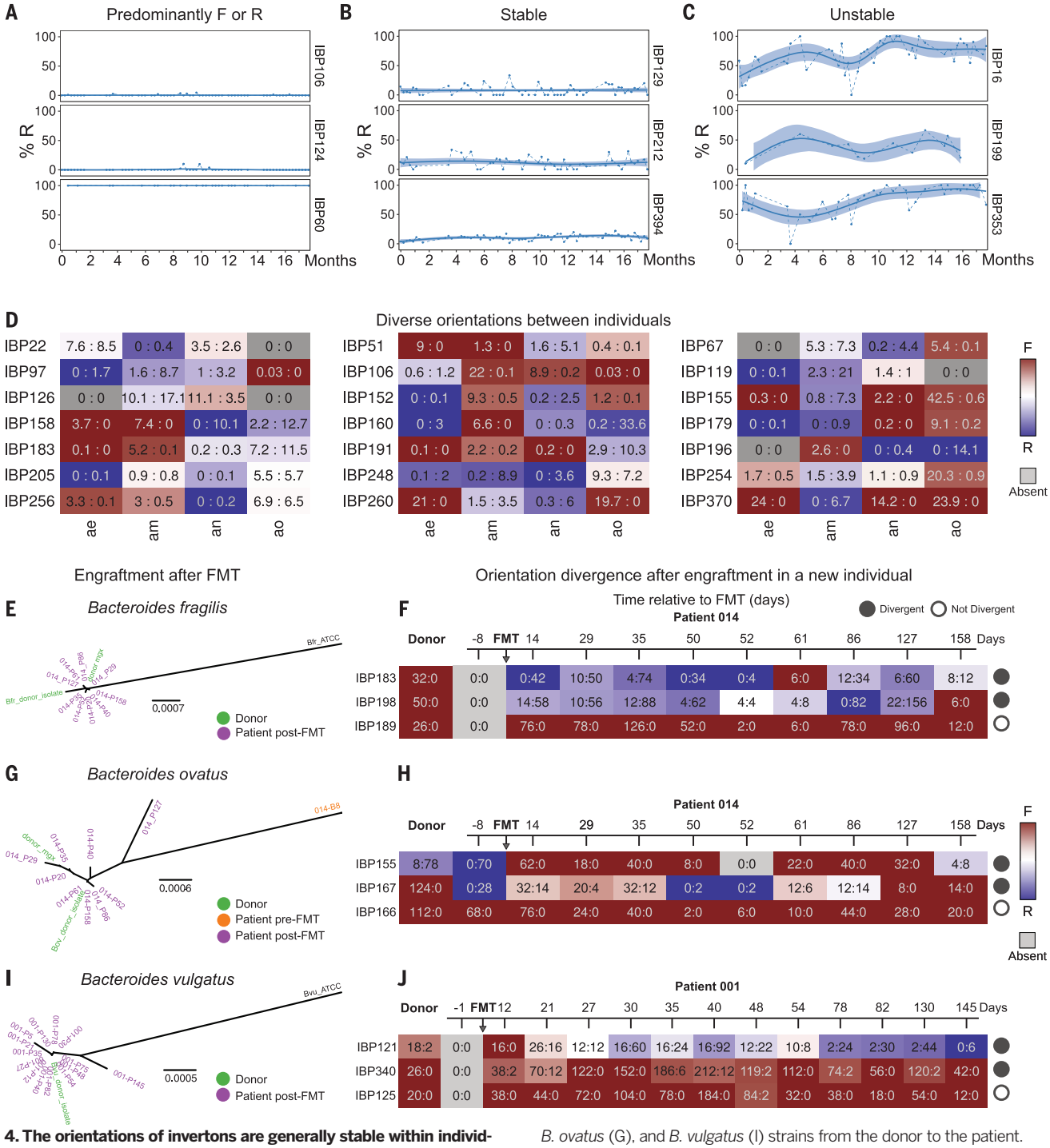

Fig. 4. The orientations of invertons are generally stable within individuals but divergent between individuals. (**A**) Examples of invertons from donor "am" found in the forward (F: IBP106, IBP124) or reverse (R: IBP60) orientation. (**B**) Examples of invertons from donor "am" (IBP129, IBP212, and IBP394) that are longitudinally stable. (**C**) Examples of invertons from donor "am" that are longitudinally unstable (IBP16, IBP199, and IBP353). (**D**) Selected examples of invertible promoters with different orientations among four individuals ("ae," "am," "an," and "ao"). Numbers in each heat map cell represent the average counts in the F and R orientations (F:R); red and blue indicate F and R orientation, respectively. (**E**, **G**, and **I**) Phylogenetic trees produced by StrainPhlAn from metagenomic data from FMT donor (green) and patient (orange, pre-FMT; purple, post-FMT) as well as isolate genomes from the donor (green) and unrelated reference genomes from the same species (black). The phylogenetic trees demonstrate engraftment and persistence of *B. fragilis* (E), *B. ovatus* (G), and *B. vulgatus* (I) strains from the donor to the patient. Phylogenetic tree legends are the number of nucleotide substitutions per site. (**F**, **H**, and **J**) Examples of divergence of invertible promoter orientations after engraftment in a patient. Black circles denote invertible promoters whose orientation was significantly different between donor and patient after FMT. White circles denote invertible promoters whose orientation is not significantly different (Wilcoxon rank sum test, FDR $P < 0.05$). Numbers in each heat map cell represent the counts in the F and R orientations (F:R). (F) After engraftment, the orientations of IBP183 and IBP198 were reversed relative to the donor. (H) A strain of *B. ovatus* was present in Patient 14 before FMT but was outcompeted by the donor strain. After engraftment, the orientation of IBP155 and IBP167 was reversed relative to the donor. (J) After *B. vulgatus* engraftment, the orientation of IBP121 was only found in the F orientation, but over the course of 135 days, the orientation reversed.

oriented either primarily ON or OFF into media with (Erm+) or without (Erm–) erythromycin and quantified the percentage of cells in the ON or OFF orientation after 24 hours (Fig. 3D). The OFF cultures grew in Erm+ medium only after an extended lag phase relative to growth in Erm– medium, whereas ON cultures grew similarly in Erm+ and Erm– media (Fig. 3E). OFF isolates grown in Erm+ medium became predominantly ON, whereas OFF isolates grown in Erm– medium remained OFF. ON isolates remained predominantly ON in both Erm+ and Erm– media (Fig. 3D).

Finally, during serial transfers in both Erm+ and Erm– media, we monitored the ON:OFF ratio of the *cmeABC/ermG* invertible promoter in a *B. stercoris* OFF isolate that had previously been switched to ON in Erm+ medium. Over the course of 24 transfers at 1:1000 dilution, the orientation of the *cmeABC/ermG* invertible promoter remained ON in Erm+ medium but gradually drifted away from 100% ON in Erm– medium, recapitulating the in vivo observations from metagenomic data (Fig. 3F).

Although strongly favored in the presence of antibiotics, high expression of antibiotic resistance genes likely incurs a substantial fitness cost, which could explain the reversion to the OFF orientation after antibiotic treatment. Many compensatory mechanisms to maintain antibiotic resistance in the absence of antibiotics have been noted (*26*), but invertons are akin to catastrophe insurance; a certain percentage of the population is always prepared to resist future antibiotic treatment and reintroduce heterogeneity after antibiotic selection.

Dense longitudinal metagenomic data allow for a detailed view of the dynamics of invertons over time in the human gut. We analyzed a dataset of samples from 54 individuals, four of whom ("ae," "am," "an," "ao") were sampled densely over 5 to 18 months, and tracked the orientations of invertons. The F orientation is the same orientation of the inverton in the reference genome, whereas the R orientation is the opposite orientation of the inverton in the reference genome. We identified 423 invertons with sufficient coverage to track their temporal dynamics in these individuals. Of these, 322 were predominantly found in one orientation within an individual with little or no fluctuation (mean > 95% and min > 75% for either the F or R orientation) (Fig. 4A); the orientations of 59 were relatively stable (max-min %R ≤ 50%) within an individual (Fig. 4B), whereas the orientations of 42 were unstable (max-min %R > 50%) within an individual (Fig. 4C and table S13).

Although the orientations of 90% of invertons in the same individual were relatively stable over time, the orientations between individuals varied extensively (Fig. 4D and fig. S10). The mean %R orientation of 214 out of 423 of the invertons varied by more than 50% between individuals. In 122 examples, averaging across time, the inverton was predominantly (>95%) in the F orientation in at least one individual and predominantly (>95%) in the R orientation in another. Additionally, 119 out of 238 invertons were significantly different (Kruskal-Wallis H test, FDR $P < 0.05$) between the four individuals for whom we had dense longitudinal metagenomic data. The differences in the orientations of invertons between individuals could be explained by divergent selective forces between individuals, different optimal orientations between strains, or stochastic variation in orientation.

Sculpted by the individual's diet, lifestyle, immune response, and genetics, the gut of every individual is a distinctive environment for resident bacteria. We observed the influence of the individual on the orientations of invertons in a cohort of patients with ulcerative colitis who were the recipients of FMT from healthy donors. The source of the fecal microbiota was donor "am," whose longitudinal metagenomic data were analyzed above. Therefore, we could monitor the orientations of invertons from the same strain for 18 months in a healthy donor and up to 5 months in the patients.

First, we identified strains from the donor that engrafted into the patient's microbiome. To identify engraftment, we found cases where the same strain was present in the donor and in at least one patient after FMT, but absent in the same patient(s) before FMT (Fig. 4, E, G, and I, and fig. S11). We found three high-abundance species with invertons from the donor that engrafted in patients: *B. fragilis*, *B. vulgatus*, and *B. ovatus*.

Then, we compared the orientations of invertons from the donor and patient after engraftment and found that the orientations of 42.8% of invertons (48/112) diverged from the donor orientation (Wilcoxon rank sum test, FDR $P < 0.05$) (Fig. 4, F, H, and J, and fig. S12). In *B. fragilis*, two invertons (IBP183 and IBP198) engrafted in the opposite orientation of the donor strain and remained predominantly (87.2% and 87.1%) in the R orientation, but drifted toward F near the end of the sampling (Fig. 4F). For *B. ovatus*, a strain existed in Patient 014 before FMT but was replaced by the donor *B. ovatus* strain (Fig. 4G; compare orange to purple). IBP155 invertons from both the donor strain and pre-FMT strain were predominantly (90.1% and 100%) in the R orientation, whereas the newly engrafted strain was oriented entirely in the F orientation and remained in the F orientation over the course of sampling (Fig. 4H). In *B. vulgatus*, an inverton was initially present completely in the F orientation but over the course of 145 days completely reversed to the R orientation (Fig. 4J, IBP121). In addition to the invertons that reversed their orientations, we also identified examples of invertons that maintained their orientations (Fig. 4F, IBP189; Fig. 4H, IBP166; Fig. 4J, IBP125).

Our findings highlight the role of invertons in host-microbe coexistence. Genes regulated by invertons were highly enriched for products located on the exterior of the cell where they are exposed to the host immune system and phages, indicating that they may be primary targets for selection that are beneficial for gut commensals to diversify their surface architectures or essential processes, such as antibiotic resistance, whose expression has a high fitness cost. The high prevalence of antibiotic resistance genes regulated by invertons containing promoters suggests that this is an example of bet-hedging (*27*). This could lead to longer persistence of antibiotic resistance genes in a microbial community, further increasing the burden to combat antibiotic resistance. Our results indicate that in the human gut, invertons help bacterial populations regain heterogeneity after bottlenecks encountered during colonization of a new host or severe perturbations. Overall, our study provides insights into a mechanism allowing adaptive tradeoffs in bacteria that have evolved to successfully colonize host-associated niches.

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ACKNOWLEDGMENTS

We thank T. Poon, T. Vatanen, and M. Groussin for their assistance in obtaining samples and data; T. Reimels for comments on the manuscript; and the Microbial Omics Core of the Broad Institute and the Broad Technology Labs for their assistance with DNA

extraction, library preparation, and sequencing. The FMT research was approved by the Committee on Human Research in the Medical Sciences (CHRMS) (CHRMS 15-373). **Funding:** Supported by NIH grants DK043351 and AT009708, the Crohn's and Colitis Foundation of America, the Juvenile Diabetes Research Foundation, and the Center for Microbiome Informatics and Therapeutics at MIT (R.J.X.). A.B.H. is a Merck Fellow of the Helen Hay Whitney Foundation. **Author contributions:** Conceptualization, X.J., A.B.H., T.D.A., H.V., A.C.T., E.J.A., and R.J.X.; methodology, X.J., A.B.H., D.R.P., C.T.C., and T.D.A.; software, X.J. and A.B.H.; validation, X.J. and A.B.H.; formal analysis, X.J., A.B.H., D.R.P., C.T.C., and T.D.A.; investigation, X.J., A.B.H., and T.D.A.; resources, M.P., P.L.M., and J.C.; data curation, X.J., A.B.H., and T.D.A.; writing (original draft preparation), X.J. and A.B.H., writing (review and editing), X.J., A.B.H., H.V., A.C.T., E.J.A., and R.J.X., visualization, X.J. and A.B.H.; supervision, H.V., A.C.T., E.J.A., and R.J.X.; project administration and funding acquisition, E.J.A. and R.J.X. **Competing interests:** E.J.A. is a co-founder and shareholder of Finch Therapeutics. J.C. consults for Finch Therapeutics. P.L.M. is currently employed by Takeda Pharmaceuticals International. **Data and materials availability:** Data used in the study are available from the NCBI. Isolate genomes: PRJNA496358. Dense longitudinal metagenomic data: PRJNA503484, FMT metagenomic data: PRJNA474024. PhaseFinder is available from GitHub: https://github.com/XiaofangJ/PhaseFinder.

SUPPLEMENTARY MATERIALS

19 June 2018; accepted 3 December 2018
10.1126/science.aau5238