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Switching of Flagellar Motility in *Helicobacter pylori* by Reversible Length Variation of a Short Homopolymeric Sequence Repeat in *fliP*, a Gene Encoding a Basal Body Protein

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The genome of *Helicobacter pylori* contains numerous simple nucleotide repeats that have been proposed to have regulatory functions and to compensate for the conspicuous dearth of master regulatory pathways in this highly host-adapted bacterium. *H. pylori* strain 26695, whose genomic sequence was determined by The Institute for Genomic Research (TIGR), contains a repeat of nine cytidines in the *fliP* flagellar basal body gene that splits the open reading frame in two parts. In this work, we demonstrate that the 26695_{C9} strain with a split *fliP* gene as sequenced by TIGR was nonflagellated and nonmotile. In contrast, earlier isolates of strain 26695 selected by positive motility testing as well as pig-passaged derivatives of 26695 were all flagellated and highly motile. All of these motile strains had a C_8 repeat and consequently a contiguous *fliP* reading frame. By screening approximately 50,000 colonies of 26695_{C9} for motility in soft agar, a motile revertant with a C_8 repeat could be isolated, proving that the described switch is reversible. The *fliP* genes of 20 motile clinical *H. pylori* isolates from different geographic regions possessed intact *fliP* genes with repeats of eight cytidines or the sequence CCCCACCC in its place. Isogenic *fliP* mutants of a motile, C_8 repeat isolate of strain 26695 were constructed by allelic exchange mutagenesis and found to be defective in flagellum biogenesis. Mutants produced only small amounts of flagellins, while the transcription of flagellin genes appeared unchanged. These results strongly suggest a unique mechanism regulating motility in *H. pylori* which relies on slipped-strand mispairing-mediated mutagenesis of *fliP*.

Helicobacter pylori, the causative agent of gastritis and ulcer disease in humans, relies on its high motility in viscous environments to colonize and persist in the human stomach (14, 18). *H. pylori* carries a unipolar bundle of sheathed flagella (19). Relatively little is known about the regulation of flagellar biosynthesis in *H. pylori* (32, 33). The whole genome sequence of *H. pylori* strain 26695 (determined by The Institute for Genomic Research [TIGR]) has revealed a conspicuous lack of regulatory elements that are present in other eubacterial species (36). *H. pylori* does not possess a homolog of the *flhCD* master operon which is at the top of the regulatory hierarchy coupling cell division and motility functions in *Salmonella enterica* serovar Typhimurium, *Bacillus subtilis*, and other eubacteria (23). *H. pylori* also appears to lack a homolog of the *flgM* gene that in other eubacteria codes for an antagonist of the flagellar sigma factor, σ^{28} (15). Except for the gene coding for the major *H. pylori* flagellin, *flaA*, most flagellar genes of *H. pylori* are governed by σ^{54}- or σ^{70}-dependent promoters (28, 33, 34). Taken together, the available data suggest that the regulation of flagellar biogenesis and motility differs considerably between *H. pylori* and other bacteria.

The complete genome sequences of *H. pylori* strains 26695 and J99 contain close to 30 genes with simple sequence repeats (dinucleotide repeats or homopolymeric tracts), either within the upstream regulatory regions or within the coding sequences (2, 36). Because of the abundance of such sequence repeats, slipped-strand mispairing has been suggested to be involved in the control of gene expression in *H. pylori* (31). Simple nucleotide repeats are mutational hot spots because they reduce the fidelity of both DNA replication and transcription. Changes of repeat length due to slipped-strand mispairing (insertion or deletion of repeat units) and consequent disruption of open reading frames by premature stop codons occur at a much higher rate than mutations in other areas of the chromosome. Slipped-strand mispairing plays a regulatory role, on both transcriptional and translational levels, in several other bacteria, mainly for the variation of surface-associated proteins and related structures such as fimbriae, capsules, or lipopolysaccharides (13, 25, 39; for a review, see reference 38). Recently, slipped-strand mispairing within fucosyltransferase genes of *H. pylori* has been demonstrated to play a role in the variation of lipopolysaccharide O-specific side chains (4, 40). Among the potentially phase-variable genes of *H. pylori*, there is also one motility-associated gene, *fliP*, the product of which is involved in the flagellar export apparatus of eubacteria (27, 31).

In *H. pylori* strain 26695, whose genome has been sequenced completely, *fliP* contains a repeat of nine cytidines (C_9) within its coding region (36). This repeat causes a frameshift and splits the gene in two parts (HP0684 and HP0685). In the second complete genome sequence of an *H. pylori* strain (J99), *fliP* is a contiguous open reading frame with a C_8 repeat (2).

We therefore wanted to know if the C_9 repeat in *fliP* might in fact be involved in motility regulation in *H. pylori* and if the motility phenotype of the organism is dependent on expression of a full-length FliP protein. We have studied the *fliP* locus in different motile and nonmotile variants of strain 26695 and in

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different clinical isolates of *H. pylori*. In addition, we constructed and characterized isogenic *fliP* mutants of different *H. pylori* strains. The results make a convincing case for slipped-strand mispairing within the *fliP* locus as a novel regulatory feature of *H. pylori* motility.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *H. pylori* strains N6 (11) and 88-3887, a motile pig-passaged variant of *H. pylori* 26695 (36), and the mouse colonizing strain SS1 (21) were used for the construction of *fliP* mutants. *fliP* sequences were determined for various motile and nonmotile variants of 26695, for N6, SS1, the nonmotile strain Tx30a (22), and for 20 clinical isolates from Germany, South Africa, and Singapore (1, 35). Culture conditions for *H. pylori* strains were as described elsewhere (34).

DNA manipulation, PCR, and nucleotide sequencing. DNA manipulations were done according to standard protocols (30). *H. pylori* genomic DNA for sequence determination was prepared with a QiaAmp tissue kit (Qiagen Inc., Hilden, Germany). Plasmid DNA was purified with the Qiagen Midi column plasmid purification kit. DNA restriction fragments or PCR products were purified from agarose gels with a QiaQuick DNA purification kit (Qiagen). Nucleotide sequences were determined by direct sequencing of PCR products generated using the primers OLHPFliP1 (CCTCATTTGCCCTTTAATATGC) and OLHPFliP2 (GGCAGAGAAATCATTACAGG). PCR consisted of 35 cycles as follows: denaturation, 94°C for 1 min; annealing, 50°C for 1 min; and extension, 72°C for 1 min; 75-ng aliquots of PCR products purified using the QiaQuick PCR purification kit (Qiagen) were used in cycle sequencing reactions from both strands with an ABI Prism dye terminator cycle sequencing kit (Applied Biosystems), using the same primers and independent PCR products for each strand. Sequences were aligned using Seqlab and Pileup from the Wisconsin Package, version 9.1 (Genetics Computer Group, Madison, Wis.). All sequences were reduced to a common length of 480 nucleotides.

Construction of isogenic *fliP* mutants of *H. pylori*. The complete *fliP* gene from the motile 26695 variant 88-3887 (*fliP*$_{C8}$) including additional upstream and downstream sequences (1,622 bp) was amplified by PCR with the primers OLHPFliP3s (TATGGATCCCATAACCTTTAGGGTCAGC) and OLHPFliP4s (TTAGGATCCGACTTTTGGTATTAGCAGC), which both contained *Bam*HI sites, and cloned into vectors pILL570 (20) and pUC18 cut with *Bam*HI to give plasmids pCJ53 and pCJ51, respectively. Subsequently, the cloned *fliP* gene was disrupted by insertion of a cassette that contains a kanamycin resistance gene (*aphA-3* [37]). The cassette was inserted in two different positions. In plasmid pCJ55, a direct derivative of pCJ53, the cassette was introduced into a natural *Eco*RI restriction site at nucleotide position 360 of the *fliP* locus in *H. pylori* KE26695, which is 151 nucleotides downstream of the C_8 repeat and 96 nucleotides downstream of the stop codon. To construct a plasmid where the insertion was located exactly at the same position of the premature stop codon in C_9 strains, the following strategy was used. Inverse PCR with primers OLHPFliP5s (TTAAGATCTCTATGATACAGGGATTAAGC) and OLHPFliP6s (ATTAG ATCTCGAGACTAAAATTTGAGTGG) and plasmid pCJ51 (*fliP* insert in pUC18) as the template was used to generate a 50-bp deletion in *fliP* and to introduce a *Bgl*II restriction site at this deletion site just 18 nucleotides downstream of the C_8 repeat. The deletion (nucleotides 229 to 279 of *fliP*) includes the stop codon generated by the *fliP*$_{C9}$ locus in 26695 (the TIGR strain). The *aphA-3* cassette (cut from pILL600 with *Bam*HI) was ligated into this construct, yielding plasmid pCJ57. Since *fliP* is not part of an operon and the two genes downstream of *fliP* are transcribed in the opposite direction of *fliP*, polar effects of these disruptions were extremely unlikely. Nevertheless, in both plasmids pCJ55 and pCJ57, the *aphA-3* cassette, which has a strong promoter and no transcription terminator, was inserted in the same transcriptional orientation with respect to *fliP*, further reducing the possibility of polar effects. Plasmids pCJ55 and pCJ57 were used to generate allelic replacement mutants of *H. pylori* strains SS1 and N6 and the motile 26695 variant 88-3887 by natural transformation. Natural transformation of *H. pylori* was performed as described elsewhere (6). After natural transformation, the bacteria were grown on nonselective plates for a period of 24 h and then transferred to plates containing kanamycin (20 mg/liter). Recombinant colonies were selected after 3 to 5 days of growth. The correct genotype of the kanamycin-resistant mutants was verified by PCRs with primers binding to the target gene and primers binding to the *aphA-3* cassette as previously described (34) (data not shown).

SDS-PAGE and Western blotting. Whole-cell lysates of *Helicobacter* cells were obtained by sonication, while flagellar filament proteins were partially purified by mechanical shearing and ultracentrifugation as described elsewhere (12). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting were performed as described elsewhere (32). The blots were incubated with a mixture of antisera raised against purified recombinant FlaA and FlaB flagellins of *H. pylori*, each diluted 1:1,500. Bound antibodies were visualized with a peroxidase-coupled goat anti-rabbit antibody (diluted 1:3,000; Jackson Biologicals Laboratory, West Grove, Pa.).

Electron microscopy and motility testing. Transmission electron microscopy of negatively stained bacteria was performed as described elsewhere (17). Motility testing of *H. pylori* strains was performed either in wet mounts or in 0.3% motility agar plates as described elsewhere (7, 17). Motility plates were incubated for 5 days.

Preparation of RNA from *H. pylori*. RNA from *H. pylori* was prepared by the RNEasy Midi-Prep procedure (Qiagen) or using a CsCl centrifugation method described by Spohn and Scarlato (33). RNA was prepared from bacteria grown on two blood agar plates for 24 h or from 25 ml of liquid culture grown to an optical density at 600 nm of about 1.0 (mid-log phase, approximately 10^{10} bacteria). RNA slot blotting was done with a Bio-Dot slot blotting apparatus (Bio-Rad). The amount of specific mRNA was detected by hybridization with digoxigenin (DIG)-labeled DNA probes (*flaA*, *flaB*, *flgE*, and *fliP*; PCR-generated fragments; probes were generated with a DIG-labeling kit from Boehringer/Roche); 2-μg aliquots of RNA were analyzed on the blots. In all strains, the hybridization signal with a *fliP* probe was too weak to be evaluated, even when 5 μg of RNA was used. Control hybridizations using the four different probes on an *Escherichia coli* DH5α RNA gave no detectable background signal. The sequences of the primers used to generate the probes by PCR are available upon request.

Nucleotide sequence accession number. The nucleotide sequences described in this paper have been submitted to the GenBank database (accession no. AJ404379 to AJ404400).

RESULTS

***H. pylori* 26695$_{C9}$ (the TIGR strain) is nonmotile and lacks flagella.** Because the genome sequence of *H. pylori* 26695$_{C9}$ does not contain a full-length *fliP* gene, we analyzed whether this strain carried flagella and was motile. *H. pylori* 26695$_{C9}$ was studied by electron microscopy, and no flagella were detected (data not shown). Bacteria were completely nonspreading in motility agar, and no motility was visible by direct microscopy of wet mounts (Fig. 1).

Different *fliP* genotypes in motile and nonmotile variants of strain 26695. Previous studies have shown that motility is essential for the ability of *H. pylori* and other *Helicobacter* spp. to colonize the gastric mucosa (3, 9, 16). It was therefore surprising that a virulent isolate of *H. pylori* was nonmotile. *H. pylori* 26695 was received by one of us in 1986. It was noted that colonies of highly motile and nonmotile bacteria could be identified in soft agar, and both variants were isolated and frozen (8).

To clarify whether the motility of different 26695 variants depended on the length of the *fliP* repeat sequence, a 642-bp fragment of *fliP* that contained the homopolymeric repeat was amplified by PCR and sequenced directly from both strands for 26695$_{C9}$ as well as several motile and nonmotile variant strains (Table 1). 26695$_{C9}$ contained a C_9 repeat and a premature stop codon in *fliP* 53 nucleotides downstream of the repeat, as reported by Tomb et al. (36) (Fig. 2). An original motile variant (26C) that had been selected because it formed large spreading colonies in motility agar had eight cytidine residues and a continuous *fliP* gene, while a nonmotile variant (26B) had nine cytidines (Table 1; Fig. 2). We also analyzed six strains each that had been obtained from initial motile or nonmotile variant strains by repeatedly passaging them in vitro without selection for or against motility (up to 10 passages). No changes in the repeat length were detectable in those passaged strains. Three strains (for example, strain 88-3887) that had been reisolated from piglets that had been experimentally infected with the original (mixed) *H. pylori* 26695 were fully motile and possessed the *fliP*$_{C8}$ genotype.

Isolation of motile revertants of 26695$_{C9}$. To demonstrate that the switch from motile (C_8) to nonmotile (C_9) was reversible, we isolated a motile revertant of 26695$_{C9}$. The bacteria were diluted in motility agar and poured into petri dishes. Approximately 50,000 colonies were screened before a spreading colony was detected. The spreading colony was isolated and purified through a second round of motility testing. As expected, the motile revertant (26695-R1) had a C_8 repeat. A second revertant (26BH) was isolated from strain 26B (C9) by growing a stab preparation of 26B in motility agar for 4 days,

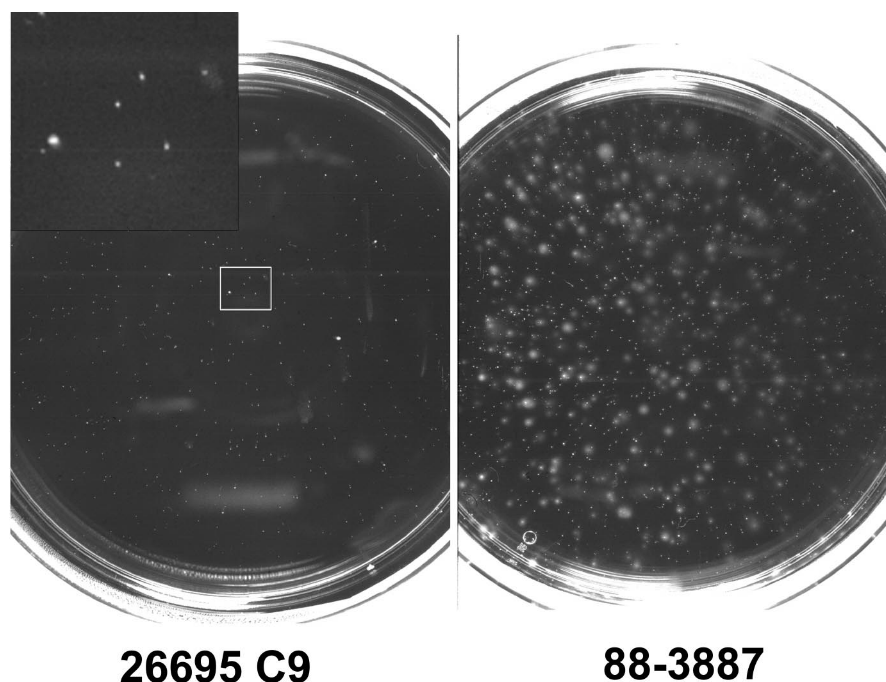

FIG. 1. Motility tests of *H. pylori* 26695_{C9} and the pig-passaged variant, 88-3887. The nonmotile 26695_{C9} strain shows only pinpoint colonies without spreading, while the majority of 88-3887 colonies have a spreading phenotype. The inset in the upper left corner shows a magnification of the area of the plate marked by the square.

swabbing the edge of the streak, and culturing that in broth. That broth produced 117 motile colonies (of 152 total), one of which was colony purified and became 26BH. Like 26695-R1, 26BH had a C_8 repeat.

***fliP* sequence comparison of different *H. pylori* strains.** To determine if the poly(C) repeat within *fliP* was conserved between different *H. pylori* strains, we evaluated *fliP* sequences (480 nucleotides) from 17 clinical isolates of *H. pylori* and from four widely used laboratory strains of *H. pylori* (SS1, NCTC11637, NCTC11639, and Tx30a [Table 1; Fig. 2]). With the exception of strain Tx30a, which, consistent with previous reports (7), was nonmotile and lacked flagella, all strains analyzed were motile and carried flagella. Strain Tx30a possessed a C_7 stretch instead of the C_8 sequence of the motile wild-type strains. All motile strains had a continuous *fliP* gene; 13 out of the 22 motile strains had an adenine instead of the fifth cytidine within the repeat (CCCCACCC). All five strains from Asia had the CCCCACCC-type allele, while only one out of six strains from the Cape Coloured population in South Africa had that allele.

Construction and characterization of isogenic *fliP* mutants. To prove that the premature stop of FliP translation in the strains with a $fliP_{C9}$ or $fliP_{C7}$ sequence was sufficient to cause the loss of motility and flagellation observed in 26695_{C9} and Tx30a, isogenic *fliP* mutants of three different motile *H. pylori* strains (the mouse-colonizing strains SS1 and N6 and the piglet-passaged motile variant 88-3887 of strain 26695) were constructed by disruption of *fliP* with an *aphA-3* cassette at two different positions, both close to the position of the premature stop codon in $fliP_{C9}$ strains (Fig. 2A). For each of the wild-type strains, two different *fliP* mutants were constructed by natural transformation with pCJ55 or pCJ57. Prior to transformation, the wild-type strains were again checked for motility and the presence of flagella to ensure that motility had not accidentally been lost by repeated in vitro passage.

The *fliP* region of all mutant strains obtained by allelic exchange mutagenesis was resequenced to exclude that the repeat length had changed during the mutant selection process. All mutants had retained the C_8 repeat and had integrated the *aphA-3* cassette into their genome at the predetermined sites. In all mutant strains, independent of the parent strain, the sequenced part of *fliP* was identical to the 26695 *fliP* sequence, because during the double-crossover event, the wild-type *fliP* sequences had been replaced by the 26695 sequences flanking the *aphA-3* cassette in the suicide plasmids. Both types of mutants in the different *H. pylori* strains were characterized for motility and the presence of flagella, and flagellin expression was checked by Western blot analysis. The phenotypes of both types of *fliP* mutants (pCJ55 and pCJ57) were identical in all strains and indistinguishable from the phenotype of 26695_{C9}. All *fliP* mutants failed to form flagella as determined by transmission electron microscopy and were nonmotile.

Whole-cell lysates and sheared flagellar material of selected wild-type strains with different *fliP* genotypes and isogenic *fliP* mutant strains were analyzed for the presence of flagellins by Western blotting with antisera raised against *H. pylori* FlaA and FlaB flagellins (Fig. 3). Flagellin synthesis in 26695_{C9} was very low compared to motile *H. pylori* wild-type strains, but FlaA was present in whole-cell lysates and, in much lower amounts, in sheared material. To exclude that free flagellin is released into the medium by the nonmotile strains, supernatants of strains grown in liquid culture were precipitated with trichloroacetic acid and analyzed; they did not contain significant amounts of flagellin (data not shown).

Transcriptional analyses of flagella-associated genes in nonmotile *H. pylori fliP* mutant strains and in motile wild-type strains. Transcription of the *flaA* and *flaB* flagellin genes and the hook gene, *flgE*, was measured by RNA slot blot hybridization in *H. pylori* 26695_{C9}, the motile variant of 26695 (88-3887; $fliP_{C8}$), an isogenic *fliP* mutant of 88-3887, and the N6 wild-type strain. In all strains with an interrupted *fliP* gene, *flaA*-, *flaB*-, and *flgE*-specific mRNAs were present in about the

TABLE 1. *H. pylori* strains characterized in this study

Strain	Origin or description	*fliP* genotype	Presence of flagella and motility
26695 variants			
26695_{C9}	United Kingdom	C_9	No
88-3887	26695, after 3 pig passages	C_8	Yes
26B	Initial nonmotile subclone of 26695	C_9	No
26BH	Motile revertant of 26B	C_8	Yes
26C	Initial motile subclone of 26695	C_8	Yes
26695-R1	Motile revertant of 26695_{C9}	C_8	Yes
Laboratory strains			
Tx30a	United States	C_7	No
N6	France	CCCCACCC	Yes
NCTC11637	Australia	CCCCACCC	Yes
NCTC11639	Australia	C_8	Yes
SS1	Australia	CCCCACCC	Yes
Asia			
RE7003	Singapore	CCCCACCC	Yes
RE8029	Singapore	CCCCACCC	Yes
RE8038	Singapore	CCCCACCC	Yes
RE12001	Singapore	CCCCACCC	Yes
RE12004	Singapore	CCCCACCC	Yes
South Africa			
CC1	Cape Town	CCCCACCC	Yes
CC7	Cape Town	C_8	Yes
CC26	Cape Town	C_8	Yes
CC29	Cape Town	C_8	Yes
CC48	Cape Town	C_8	Yes
CC56	Cape Town	C_8	Yes
Germany			
BO242	Bochum	CCCCACCC	Yes
BO255	Bochum	CCCCACCC	Yes
BO261	Bochum	C_8	Yes
BO265	Bochum	C_8	Yes
BO266	Bochum	CCCCACCC	Yes
BO314	Bochum	CCCCACCC	Yes

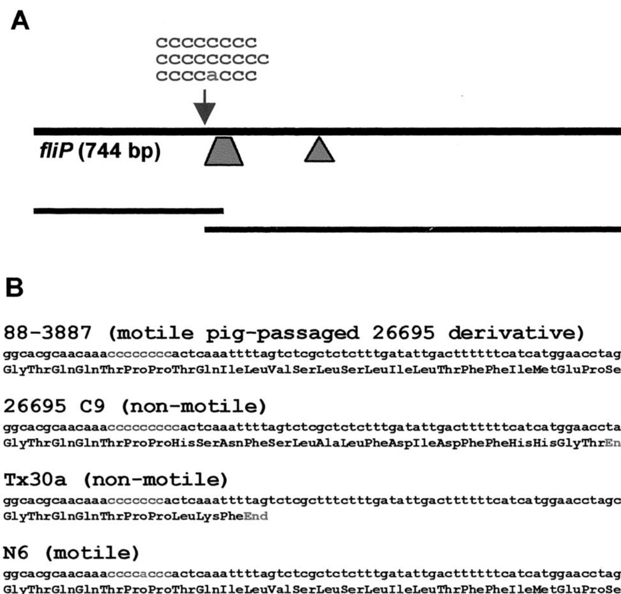

FIG. 2. (A) Schematic representation of *H. pylori fliP* and positions of the poly(C) repeat (arrow) and insertion points of the kanamycin resistance cassette in plasmids pCJ55 (triangle; insertion into *Eco*RI site) and pCJ57 (trapezoid; 50-bp deletion). (B) Nucleotide sequences and deduced translation (in three-letter code) of the region surrounding the poly(C) repeat in a motile and a nonmotile variant of *H. pylori* 26695, in the nonmotile strain Tx30a, and in *H. pylori* N6. The repeat is located 315 nucleotides downstream of the *fliP* start codon.

same amounts as in the motile wild-type strains (Fig. 4). These data ruled out the possibility that the observed profound reduction in flagellin in these strains was due to a downregulation of flagellin gene transcription.

DISCUSSION

***fliP* genotypes and motility in *H. pylori* 26695.** The data presented here show that *H. pylori* can use slipped-strand mispairing-mediated frameshifting in *fliP* to switch the formation of flagella and hence motility off and on. *fliP* encodes a component of the flagellar basal body and was not previously known to be involved in flagellar regulation of any bacterial species (24). *fliP* can be reversibly inactivated by addition or deletion of a single cytidine which results in a frameshift and introduces an early stop codon. The consequence of *fliP* inactivation is a shutdown of flagellar assembly. The observed reduction in the expression of flagellins was not due to transcriptional regulation, because mRNA for major structural flagellar components was present in unchanged amounts in *fliP* knockout mutants and repeat length variants.

It has been known for a long time that *H. pylori* loses its motility after prolonged in vitro passage, but the mechanisms responsible for this loss of motility were not known. The *H. pylori* genome contains relatively few regulatory genes. A striking example is the absence of master regulatory genes (*flhCD* and *flgM*) from the flagellar regulatory cascade that have central roles in other bacterial species. In agreement with this, a lack of feedback mechanisms coupling flagellar assembly to the expression of late flagellar genes has been observed in *H. pylori* hook (*flgE*) and flagellin (*flaA* and *flaB*) mutants (28, 34). The

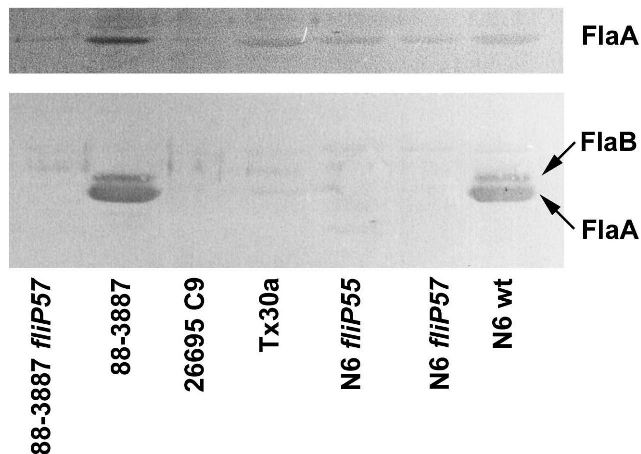

FIG. 3. Western blot analysis of flagellin expression in *H. pylori* strains with different *fliP* genotypes. (Top) Whole-cell lysates; (bottom) partially purified flagella. Material prepared from equal numbers of bacteria was loaded in each lane of the gel. Blots were developed with antisera raised against recombinant *H. pylori* flagellins. wt, wild type.

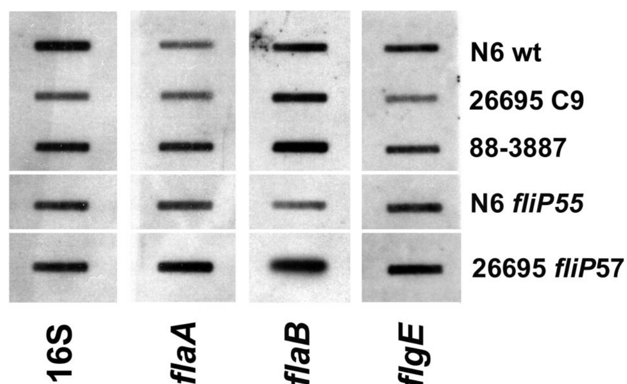

FIG. 4. Slot blot hybridization of RNA isolated from *H. pylori* strains with different *fliP* genotypes with DNA probes specific for 16S rRNA (16S) and *flaA*-, *flaB*-, or *flgE*-specific mRNA. Each slot contained 2 μg of total RNA. wt, wild type.

switching mechanism described here may compensate, at least in part, for the lack of other regulatory elements.

The *fliP*-based switching mechanism appears to be relatively specific to *H. pylori*. A manual search of all available *fliP* sequences in GenBank for homopolymeric tracts or dinucleotide repeats was performed. No homopolymeric tract was present in the *fliP* gene of *Campylobacter jejuni*, whose flagellar system is otherwise quite similar to that of *H. pylori*, or the *fliP* genes of any other bacterium with the notable exception of *Borrelia burgdorferi*, where the potentially frameshiftable sequence T_9AT_6 is located only few nucleotides downstream of the start codon of *fliP* (GenBank accession no. L75945). It is tempting to speculate that *B. burgdorferi*, which, like *H. pylori*, has very few regulatory genes (e.g., only two two-component signal transduction systems) may employ a similar strategy to switch off motility. Indeed, frameshifts in a motility-associated gene have only recently been shown for the first time to be involved in switching of flagellar biogenesis. Park et al. (29) showed that a nonmotile variant of *Campylobacter coli* UA585 had an interrupted *flhA* gene, which was due to a length change in a short homopolymeric T repeat. Motile revertants had corrected the repeat length and restored an intact *flhA* gene. No repeat was present in *C. jejuni flhA* or in *H. pylori flhA* (formerly *flbA*) (34).

***fliP* mutants.** The hypothesis that the nonmotile phenotypes of *H. pylori* strains 26695 and Tx30a are caused by the frameshifts in *fliP* was verified by the construction of isogenic *fliP* knockout mutants of different motile *H. pylori* strains. Polar effects of the cassette mutagenesis were extremely unlikely, because *fliP* in *H. pylori* (unlike in salmonellae) is not part of an operon. The two genes located downstream of *fliP* are transcribed in the opposite direction. The phenotype of these mutants was indistinguishable from that of 26695_{C9}.

FliP and flagellar assembly. In salmonellae, FliP is an early flagellar protein on the second hierarchical level of flagellar biogenesis and under the direct control of the *flhCD* master operon, which is not present in *H. pylori* (27). FliP, of which in salmonellae there are about five subunits per flagellum, is considered a component of the flagellar type III export system in the flagellar basal body (10). It is highly likely that *fliP* mutants are no longer able to export flagellar components (27). In the *H. pylori* nonmotile wild-type strains as well as the isogenic *fliP* mutants, small amounts of flagellin were detected in the cytoplasmic fraction of the bacteria, showing that translation still takes place. In contrast to what was described for *H. pylori flgE* mutants (28), there was no accumulation of intracellular flagellin, suggesting that flagellin stability is reduced in *fliP* mutants.

Frequency of motility switching. The switching frequencies of the motility phenotype had previously been determined to be 1.6×10^{-4} for the motile-to-nonmotile switch and less than 10^{-7} for the inverse event (8). We could not measure the rate of the *fliP* off switch, because when motile strains of *H. pylori* are tested in soft agar, many colonies appear nonmotile (such as the pinpoint colonies in the left panel of Fig. 1). Most of these either still express flagellins or, if they do not, still have a C_8 genotype (data not shown). Thus, frameshifting in *fliP* does not seem to be the only mechanism responsible for loss of motility in *H. pylori*. With more than 50 genes involved in motility and flagellar biosynthesis, there are many different possible events that could cause a nonmotile phenotype. We succeeded in detecting the *fliP* on switch. Screening of a $fliP_{C9}$ strain for back-mutation to C_8 yielded two independent revertants with two different screening approaches. However, this event was so infrequent (1 revertant in 50,000 colonies screened) that it was impossible to determine an exact switching frequency.

Since nonmotile mutants of *H. pylori* are not able to colonize in animal models, a strong selective pressure for motility must favor a motile phenotype in vivo, as has previously been shown in animal experiments (8). On the other hand, loss of this pressure—such as when bacteria are grown in vitro—leads to a frequent loss of this energy-consuming property. It is not clear which role this propensity for a relatively frequent switch to a nonmotile phenotype by mutation in *fliP* or by other mechanisms might play in vivo. It is conceivable that there is a niche for a proportion of the *H. pylori* bacteria where motility is no longer needed. In certain parts of the stomach mucosa not directly exposed to the shedding forces of mucus production or peristalsis, the energy-saving loss of motility might be advantageous for a lifestyle of low nutrient requirements, continuous slow growth, and tight adherence to cells.

Sequence polymorphisms and recombination in *fliP*. Thirteen out of 22 motile strains studied had an adenosine residue in the fifth position of the repeat. This mutation would greatly reduce the probability of slipped-strand mispairing mutagenesis. The relevance of this polymorphism in the repeat sequence is not known. Since motility is essential for colonization, it seems unlikely that a hypermutatable nucleotide sequence that renders the motility system more susceptible to functional inactivation would occur so frequently unless this had biological significance. However, the occurrence of the CCCCACCC sequence in more than half of the strains analyzed, including all of the Asian strains, shows that the presence of the C_8 repeat is not essential, at least not in all hosts. It is conceivable that there are subgroups of strains or strains in particular hosts where loss of motility is particularly detrimental, and strains with a CCCCACCC sequence therefore have a selective advantage. Given the frequency of recombination in *H. pylori* (35), this mutation in the repeat sequence would not have to be a dead end for a given strain because the C_8 repeat could be reacquired by recombination. Analysis of the *fliP* sequences with the Homoplasy test (26) shows that *fliP* has been involved in frequent recombination events (data not shown). Finally, it has recently been shown in *Neisseria meningitidis* that the frequency of slipped-strand mispairing-mediated phase variation of capsule biosynthesis is strongly dependent on the presence or absence of the mutator gene *dam* (5). Although nothing is yet known about the occurrence of mutator phenotypes in *H. pylori*, it is possible that similar differences of mutation frequencies exist in *H. pylori*, and these might strongly affect the frequency of *fliP*-mediated motility switching.

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