The fimbriae activator MatA switches off motility in *Escherichia coli* by repression of the flagellar master operon *flhDC*

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Flagella provide advantages to *Escherichia coli* by facilitating taxis towards nutrients and away from unfavourable niches. On the other hand, flagellation is an energy sink to the bacterial cell, and flagella also stimulate host innate inflammatory responses against infecting bacteria. The flagellar assembly pathway is ordered and under a complex regulatory circuit that involves three classes of temporally regulated promoters as well as the flagellar master regulator FlhD4C2. We report here that transcription of the *flhDC* operon from the class 1 promoter is under negative regulation by MatA, a key activator of the common mat (or ecp) fimbria operon that enhances biofilm formation by *E. coli*. Ectopic expression of MatA completely precluded motility and flagellar synthesis in the meningitis-associated *E. coli* isolate IHE 3034. Northern blotting, analysis of chromosomal promoter–lacZ fusions and electrophoretic mobility shift assays revealed an interaction between MatA and the *flhDC* promoter region that apparently repressed flagellum biosynthesis. However, inactivation of *matA* in the chromosome of IHE 3034 had only a minor effect on flagellation, which underlines the complexity of regulatory signals that promote flagellation in *E. coli*. We propose that the opposite regulatory actions of MatA on *mat* and on *flhDC* promoters advance the adaptation of *E. coli* from a planktonic to an adhesive lifestyle.

**INTRODUCTION**

The coordination of transition between a motile, planktonic existence and an adherent, sessile growth mode, two common and fundamental properties of the bacterial life cycle, is critical for bacterial survival in changing environments. A common form of bacterial motility is taxis mediated by flagella, which are rotary filamentous appendages that enable rapid directional movement towards favourable niches or away from detrimental conditions. Flagella and motility enhance encounter rates of pathogenic bacteria with host cells or tissues, thus facilitating the spread of infection, and therefore flagellation is advantageous to pathogenic bacteria at the early stages of infection (Ottemann & Miller, 1997; Ramos et al., 2004). In addition, the flagellar filament can act as an adhesin, and thereby increases the frequency and intimacy of early host–pathogen interactions (Erdem et al., 2007; Girón et al., 2002; Mahajan et al., 2009; Roy et al., 2009). On the other hand, flagella are highly immunostimulatory in the host and recognized by innate immunity defences (Hayashi et al., 2001). The complex biosynthesis of flagellar filaments represents a considerable energy sink for bacteria, and hence flagellation is tightly regulated in response to environmental changes, such as temperature or nutritional shifts, or transfer to favourable conditions for avoidance of cellular immunity in the host (Soutourina & Bertin, 2003).

*Escherichia coli*, one of the first colonizers of the sterile neonatal gut, is motile and normally coexists with its vertebrate hosts as a commensal. *E. coli* pathovars cause diseases that at worst can be lethal, and they are a significant public health and economic problem (Croxen & Finlay, 2010). Uropathogenic *E. coli* (UPEC) is the predominant causative agent of uncomplicated urinary tract infections (UTIs) (Gaynes et al., 2005; Gupta et al., 1999; Warren, 1996), and exhibits a number of necessary adhesins,
including P and type 1 fimbriae, which potenti ate bacterial attachment to the urinary tract (UT) tissue compartments (Connell et al., 1996; Korhonen et al., 1986). Competitive in vivo experiments in mice have demonstrated that flagella contribute to the fitness of UPEC during colonization of the UT (Lane et al., 2005; Wright et al., 2005), and transient expression of flagella enhances bacterial ascent from the bladder to the kidneys (Lane et al., 2007b). In the kidneys, motility as well as the flagellar filament itself are apparently required for bacterial invasion into cells of renal collecting ducts (Pichon et al., 2009), which also are targets for fimbria-mediated adherence of UPEC (Korhonen et al., 1986). For newborn meningitis-associated E. coli (NMEC), which represents another extraintestinal E. coli pathogroup, the flagellar structure rather than the motility function has been shown to be relevant for bacterial association with and invasion into human brain microvascular endothelial cells (Parthasarathy et al., 2007). Similarly, flagella of diarrhoeagenic E. coli have been demonstrated to be directly involved in the adherence to epithelial cells (Girón et al., 2002; Mahajan et al., 2009; Roy et al., 2009) and mucins (Erđem et al., 2007), and also to contribute to host cell invasion (Luck et al., 2006). Thus, flagellation contributes to the pathogenicity of E. coli through motility and adhesive- or invasion-promoting properties.

To maintain the balance between the need for movement, the energy cost to the cell and the selective immune pressure directed against flagellin in the host, Gram-negative bacteria have developed regulatory networks that utilize checkpoints for control of flagellar gene expression and the process of assembly. The biosynthetic pathway and mechanisms of function of the large, complex flagellar organelle are conserved in enteric bacteria that are peritrichously flagellated. Biosynthesis relies on the coordinated and temporal expression of over 60 genes organized in more than a dozen operons (Parthasarathy et al., 2007). Similarly, flagella of diarrhoeagenic E. coli have been demonstrated to be directly involved in the adherence to epithelial cells (Girón et al., 2002; Mahajan et al., 2009; Roy et al., 2009) and mucins (Erđem et al., 2007), and also to contribute to host cell invasion (Luck et al., 2006). Thus, flagellation contributes to the pathogenicity of E. coli through motility and adhesive- or invasion-promoting properties.

The ability to adhere to surfaces is a prerequisite for bacterial colonization, e.g. in the initiation of virtually all bacterial infectious processes and in bacterial biofilm formation. To avoid simultaneous expression of motility-enhancing flagella and adhesion-promoting fimbriae, various bacteria, e.g. Proteus mirabilis, Salmonella enterica and E. coli, harbour regulatory systems for reciprocal regulation of these traits (Clegg & Hughes, 2002; Li et al., 2001; Ogasawara et al., 2011; Pesavento et al., 2008; Saini et al., 2010; Simm et al., 2004). We have previously described the common and conserved Mat (meningitis-associated and temperature-regulated) fimbria (Pouttu et al., 2001) [also called ECP (E. coli common pilus); Rendon et al., 2007], which has host cell-adhesive (Avelino et al., 2010; Blackburn et al., 2009; Lasaro et al., 2009; Rendon et al., 2007; Saldaña et al., 2009) and biofilm-promoting (Lehti et al., 2010) properties, and is a critical colonization determinant for a probiotic E. coli strain in the intestine of the infant mouse (Lasaro et al., 2009). The fimbrillin subunit MatB is an abundant surface protein on meningitis-associated IHE 3034 cells from a biofilm (Lehti et al., 2010). Expression of Mat fimbiae is influenced by growth temperature and the composition of the culture medium (Pouttu et al., 2001; Rendon et al., 2007). Our ongoing work has shown that a LuxR-type transcription factor, MatA, is directly involved, as a positive regulator acting at the transcriptional and post-transcriptional levels, in the expression of Mat fimbiae in the NMEC strain IHE 3034 (T. A. Lehti and others, unpublished results). In the present study, we identified in MatA a novel function in repression of flagellation. The results suggest a mechanism of how motility is repressed in E. coli cells adhering to epithelium or living in a biofilm.

**METHODS**

**Bacterial strains and culture conditions.** Bacterial strains and plasmids are described in Table 1. Bacteria were grown in Luria–Bertani (LB) broth at 20 or 37 °C with shaking (200 r.p.m.). When necessary, antibiotics were added at the following final concentrations: ampicillin (Ap), 100 μg ml⁻¹; chloramphenicol (Cm), 25 μg ml⁻¹; kanamycin (Km), 25 μg ml⁻¹; rifampicin (Ri), 75 μg ml⁻¹; streptomycin (Sm), 100 μg ml⁻¹; tetracycline (Tc), 12.5 μg ml⁻¹. Induction of matA or matBCDEF gene expression from the pSE808-based plasmid pMAT19 or pMAT6, respectively, was done with 5 μM IPTG.

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Table 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHE 3034-Rif</td>
<td>Rifampicin-resistant IHE 3034, O18ac: K1: H7</td>
<td>Pouttu et al. (2001)</td>
</tr>
<tr>
<td>IHE 3034-102</td>
<td>IHE 3034-Rif matA(A536C), resulting in the substitution MatA H179P</td>
<td>T. A. Lehti (2010)</td>
</tr>
<tr>
<td>IHE 3034-96</td>
<td>IHE 3034-Rif ΔmatBCDEF (+61 bp from the ATG of matB to +753 bp from the ATG of matF)</td>
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<tr>
<td>IHE 3034-79</td>
<td>IHE 3034-Sm fimA::cat sfaA::Gm fliC::sat</td>
<td>Pouttu et al. (2001)</td>
</tr>
<tr>
<td>IHE 3034-130</td>
<td>IHE 3034-Rif rrrB T1C-pflhD-lacZ (−942 to +22 bp from the ATG of fliD)</td>
<td>This study</td>
</tr>
<tr>
<td>IHE 3034-131</td>
<td>IHE 3034-Rif rrrB T1C-pflIC-lacZ (−712 to +38 bp from the ATG of fliC)</td>
<td>This study</td>
</tr>
<tr>
<td>IHE 3034-132</td>
<td>IHE 3034-102 rrrB T1C-pflhD-lacZ (−942 to +22 bp from the ATG of fliD)</td>
<td>This study</td>
</tr>
<tr>
<td>IHE 3034-133</td>
<td>IHE 3034-102 rrrB T1C-pflIC-lacZ (−712 to +38 bp from the ATG of fliC)</td>
<td>This study</td>
</tr>
<tr>
<td>MG1655-Rif</td>
<td>Rifampicin-resistant MG1655, K-12, OR: H48</td>
<td>T. A. Lehti (2010)</td>
</tr>
<tr>
<td>MG1655-102</td>
<td>MG1655-Rif matA(A536C), resulting in the substitution MatA H179P</td>
<td>T. A. Lehti (2010)</td>
</tr>
<tr>
<td>S17-1 pir</td>
<td>reca thi pro hsdR RP4-2-Tc::Mu Km::Tn7 pir</td>
<td>Simon et al. (1983)</td>
</tr>
<tr>
<td>BL21(DE3)</td>
<td>F’ ompT dcm lon hsdS(rK mK) gal ΔDE3</td>
<td>Studier &amp; Moffatt (1986)</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
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<tr>
<td>pACYC184</td>
<td>Cloning vector, TcR, CmR</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>pTAL</td>
<td>IHE 3034 matA under p tet in pACYC184</td>
<td>T. A. Lehti (2010)</td>
</tr>
<tr>
<td>pTAL2</td>
<td>MG1655 matA under p tet in pACYC184</td>
<td>M. Kuukonen (1990)</td>
</tr>
<tr>
<td>pMAT6</td>
<td>IHE 3034 matBCDEF under p trc in pSE380</td>
<td>Pouttu et al. (2001)</td>
</tr>
<tr>
<td>pBAU1</td>
<td>IHE 3034 matA in pMAL-c2x</td>
<td>P. Bauchart (1990)</td>
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Construction of single-copy lacZ reporter strains. Recombinant DNA techniques were carried out using standard protocols (Sambrook & Russell, 2001). The pflhD and pflIC regulatory regions were chosen according to Sperandio et al. (2001), and the primers used are described in Table 2. To construct suicide plasmids for integration of promoter–lacZ fusions into the chromosomal lac locus, the promoter region of pflhD (−942 to +22 bp from the translational start of fliD) and pflIC (−712 to +38 bp from the translational start of flIC) were amplified from IHE 3034 genomic DNA and cloned individually into the BamHI restriction site of plasmid pUC19-placZ, which contains the IHE 3034 lacI gene and its upstream region, as well as four rrrB T1 terminators, a promoter cloning site and a part of the promotorless lacZ gene from reporter plasmid pRS551. The resulting plasmids were digested with XbaI and SacI, and the restriction fragments containing the pflhD or pflIC promoter with lacI and lacZ flanking regions were subcloned into a pir-dependent suicide vector pCVD442. To replace the native lacZ promoter with the pflhD or pflIC promoter on the chromosome of IHE 3034-Rif or its matA(A536C)

Table 2. Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Purpose</th>
</tr>
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<tbody>
<tr>
<td>315pflhD-942F</td>
<td>CGCGGATCCACGATCGGCGGAGCTAA</td>
<td>pflhD–lacZ fusion; BamHI</td>
</tr>
<tr>
<td>241pflhD22R</td>
<td>CGCGGATCCGACGAGCTGAGGATGCG</td>
<td>pflhD–lacZ fusion; BamHI</td>
</tr>
<tr>
<td>316pflIC-712F</td>
<td>CGCGGATCCGGATGTTAGCCCGGCTGAT</td>
<td>pflIC–lacZ fusion; BamHI</td>
</tr>
<tr>
<td>243pflIC88R</td>
<td>CGCGGATCCATCGCGAGGCGGCTGTTG</td>
<td>pflIC–lacZ fusion; BamHI</td>
</tr>
<tr>
<td>191lacIF</td>
<td>TTAATGACAGTCGCGACGAC</td>
<td>Screening of promoter–lacZ fusions</td>
</tr>
<tr>
<td>035lacZr</td>
<td>GTCCGTTTATGAGACGCAAC</td>
<td>Screening of promoter–lacZ fusions</td>
</tr>
<tr>
<td>190pRS551R</td>
<td>GGCCGACTGCAAATCGGTAATC</td>
<td>Sequencing of promoter–lacZ fusions</td>
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<tr>
<td>146pflD11F</td>
<td>TATTTAATTGAAAGAACATTTG</td>
<td>pflD1 EMSA fragment</td>
</tr>
<tr>
<td>148pflD11R</td>
<td>TGTGTTTCAAGCACTCGG</td>
<td>pflD1 EMSA fragment</td>
</tr>
<tr>
<td>145pflD22F</td>
<td>CCGCGATCGAATTTACC</td>
<td>pflD2 EMSA fragment</td>
</tr>
<tr>
<td>147pflD22R</td>
<td>CTCAATTACTTAAGAATTAGTG</td>
<td>pflD2 EMSA fragment</td>
</tr>
<tr>
<td>234pflICF</td>
<td>CTTTGGCAGTGGATTCC</td>
<td>pflIC EMSA fragment</td>
</tr>
<tr>
<td>235pflICR</td>
<td>GACTTGGCCATGATTCC</td>
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<td>236pflIAF</td>
<td>AGCAAGCGTGCTTCCCG</td>
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<tr>
<td>237pflIAR</td>
<td>GAGTGAATCCGATAAAC</td>
<td>pflIA EMSA fragment</td>
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</table>
derivative, allelic exchange was performed, basically as described by Mobley et al. (1993), following a modified procedure, which includes an additional selection step in sucrose broth. In brief, suicide plasmids were transferred from the donor strain S17-1 ppir into IHE 3034-Rif or IHE 3034-102 by conjugation, and the transconjugants were selected on LB agar plates containing ampicillin and rifampicin. Sodium citrate (0.5%, w/v) was added to the agar plates during mating and during transconjugant selection. Transconjugants cultivated for 18 h in non-selective LB broth were diluted 1:200 in LB broth supplemented with 10% (w/v) sucrose and cultured for an additional 5 h (an additional sucrose selection step) before plating on LB agar plates containing 10% (w/v) sucrose but lacking NaCl. Finally, the allelic exchanges were screened by colony-PCR, and the correctly integrated single-copy promoter fusions were verified by DNA sequencing.

β-Galactosidase assay. Overnight cultures of bacteria were diluted 1:100 and grown to mid-exponential phase corresponding to OD600 0.5–0.6. Cells from 1 ml aliquots were harvested by centrifugation (13 000 r.p.m. for 2 min) and resuspended in 1 ml ice-cold Z-buffer (60 mM Na2HPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4) containing 50 mM β-mercaptoethanol, and β-galactosidase activities were determined in permeabilized whole cells as described by Miller (1972) using ONPG as substrate. An unpaired Student’s t test was performed to identify statistically significant differences. P values of <0.05 were considered to be statistically significant.

Motility assay. Tryptone motility plates (1% tryptone, 0.5% NaCl, 0.3% agar) were prepared on the day prior to use and left at room temperature overnight. Then, 200 ml LB broth (with the above supplements) was inoculated at 1:100, and bacteria were grown to OD600 0.5 at 20 °C. The culture temperature was reduced to 15 °C, and IPTG was added to a final concentration of 0.3 mM for protein induction. After 3.5 h incubation, the cells were harvested, frozen at −70 °C and suspended in column buffer [20 mM Tris/HCl (pH 7.5), 200 mM NaCl, 1 mM EDTA]. MBP and MBP–MatA were purified from sonicated cells using amyllose resin according to the manufacturer’s instructions (New England Biolabs). The eluted fractions were dialysed against 15 mM Tris/HCl (pH 7.5), 45 mM NaCl and 7.5 mM MgCl2. The protein concentrations were estimated from SDS-PAGE gels by comparing intensities of stained polypeptides with those of BSA standards of known concentration using the Tina (v2.0) image analysis program (Raytest Isotopenmessgerate GmbH).

Detection of FlfC protein by Western blot analysis. Polyclonal antiserum raised in rabbits against H7 flagella of the E. coli strain IHE 3034 was available from previous work (Westerlund-Wikström et al., 1997). Whole-cell protein samples were prepared from mid-exponential phase cultures by collecting the bacteria by centrifugation (2500 r.p.m., 10 min), washing with PBS and adjusting to equal concentrations by resuspending in PBS to OD600 0.8. Pelleted cells from 0.75 ml of the bacterial suspension were resuspended in 0.1 ml SDS-PAGE loading buffer and boiled, and samples of 5 μl were run in a 12% SDS-PAGE gel and stained with Coomassie blue as a loading control.

RNA isolation and Northern blotting. Cell aliquots were harvested during the mid-exponential growth phase (OD600 0.5–0.6) by centrifugation of 1 ml of cultures at 4 °C. The pellets were resuspended in 0.1 ml Tris-EDTA buffer containing lysosyme (400 μg ml−1), and total bacterial RNA was isolated using the RNeasy Mini kit (Qiagen) following the manufacturer’s protocol (RNeasy Mini handbook). RNA concentrations were determined based on absorbance readings at 260 nm.

For quantification of flhDC and flIC mRNAs by Northern blot analysis, 5 or 2.5 μg of total RNA from each sample and DIG-labelled RNA molecular mass markers (Roche Applied Science) were fractionated in a 1.2% agarose gel containing 0.2 M formaldehyde and 0.1 μg ethidium bromide ml−1, transferred onto a positively charged nylon membrane (Roche Applied Science) by overnight capillary transfer, and cross-linked by UV radiation. DIG-labelled ssDNA probes complementary to the flhDC or flIC genes were amplified from chromosomal DNA of E. coli IHE 3034 and labelled with PCR DIG Labeling Mix (Roche Applied Science) by linear PCR. Blots were hybridized overnight at 50 °C in a high-SDS hybridization buffer [7% (w/v) SDS, 5 × SSC, 50% (v/v) formamide, 0.1% (w/v) N-lauroylsarcosine, 2% (v/v) blocking solution (Roche), 50 mM sodium phosphate at pH 7.0], and DIG-labelled probes bound to the membrane were detected with anti-DIG–alkaline phosphatase conjugate and CSPD substrate supplied with the DIG Luminescent Detection kit (Roche Applied Science) according to the manufacturer’s instructions. Chemiluminescence was visualized by exposure on X-ray films (Agfa).

Expression and purification of MBP and MBP–MatA proteins. For MBP (malto-binding protein) expression and affinity chromatography, bacterial cultures were grown in LB broth supplemented with 0.2% (w/v) glucose and the appropriate antibiotic at 20 °C overnight. Then, 200 ml LB broth (with the above supplements) was inoculated at 1:100, and bacteria were grown to OD600 0.5 at 20 °C. For MBP (maltose-binding protein) expression and affinity chromatography, bacterial cultures were grown in LB broth supplemented with 0.2% (w/v) glucose and the appropriate antibiotic at 20 °C overnight. Then, 200 ml LB broth (with the above supplements) was inoculated at 1:100, and bacteria were grown to OD600 0.5 at 20 °C. The culture temperature was reduced to 15 °C, and IPTG was added to a final concentration of 0.3 mM for protein induction. After 3.5 h incubation, the cells were harvested, frozen at −70 °C and suspended in column buffer [20 mM Tris/HCl (pH 7.5), 200 mM NaCl, 1 mM EDTA]. MBP and MBP–MatA were purified from sonicated cells using amyllose resin according to the manufacturer’s instructions (New England Biolabs). The eluted fractions were dialysed against 15 mM Tris/HCl (pH 7.5), 45 mM NaCl and 7.5 mM MgCl2. The protein concentrations were estimated from SDS-PAGE gels by comparing intensities of stained polypeptides with those of BSA standards of known concentration using the Tina (v2.0) image analysis program (Raytest Isotopenmessgerate GmbH).

Electrophoretic mobility shift assays (EMSSAs). DNA fragments for binding assays, pflhD1 (−305 to +28 from the ATG of flhD), pflhD2 (−1009 to −285 from the ATG of flhD), pflC (−273 to +12 from the ATG of flIC), pflA (−158 to +12 from the GTG of flA), pntA (−343 to +10 bp from the GTG of matA) and pntB (−150 to +36 bp from the ATG of matB), were generated by PCR from IHE 3034 genomic DNA using the primers described in Table 2. Binding assays with different concentrations of purified MBPs and approximately 0.2 pmol of each DNA fragment were carried out in 10 μl reaction mixtures containing 10 mM Tris/HCl (pH 7.5), 1 mM EDTA, 30 mM NaCl, 5 mM MgCl2, 1 mM DTT, 0.1% (v/v) Nonidet P-40, 0.05 mg BSA ml−1 and 5 μl (v/v) glycerol. Following incubation for 30 min at room temperature, the reactions were analysed on a 6% non-denaturing PAGE gel in 0.5 × Tris/borate-EDTA buffer that was prerun for 30 min at a constant voltage of 200 V. After electrophoresis at room temperature, the DNA fragments were visualized by staining the gel with ethidium bromide and exposing to UV light.

RESULTS

Constitutive expression of MatA in E. coli IHE 3034 represses motility by preventing flagellin synthesis

Our initial observation was that overexpression of the matA regulatory gene abolished the motility of IHE 3034. Phase-contrast microscopy of IHE 3034 matA derivatives showed that the entire cell population became non-motile in LB broth when the chromosomal matA(A536C) mutation, encoding the non-functional variant MatA H179P of the Mat fimbriae regulator, was complemented in trans.
Ectopic expression of the fimbriae activator gene matA downregulates flagellation. (a) Motility phenotypes were assayed by inoculating the wild-type IHE 3034-Rif strain and matA derivatives on tryptone-based soft agar (0.3%) motility plates and incubating at 20 °C for 30 h. The matA(A536C) substitution in IHE 3034-Rif was complemented with pACYC184 derivatives carrying the matA gene from IHE 3034 or from MG1655 under the constitutive ptet promoter of the vector. The non-flagellated fliC::sat derivative of IHE 3034-Sm is shown for comparison. (b) Measurement of colony diameters on motility agar plates formed after 30 h incubation at 20 °C and after 7.5 h at 37 °C. Error bars, SD of triplicate independent measurements. (c) Western blot analysis of flagellin expression in the IHE 3034 derivatives. Bacteria were grown to mid-exponential phase in LB broth at 20 °C, and whole-cell protein samples transblotted onto a nitrocellulose membrane were left to react with anti-H7 flagellum antibodies and detected with alkaline phosphatase-conjugated secondary antibody. Coomassie blue-stained polypeptides in the whole-cell samples are shown below the Western blot as protein loading controls.

with the wild-type matA gene on plasmid pTAL1. In contrast, the vector control strain was motile, with frequent changes in direction. These observations (not shown) indicated that expression of the fimbriae regulator MatA negatively affects the function of the flagellar system. To analyse various swimming phenotypes, overnight cultures of IHE 3034 derivatives were inoculated onto tryptone-based motility plates. For comparison, the non-flagellated mutant IHE 3034-79 carrying the inactivated H7 flagellin gene fliC::sat (Pouttu et al., 2001) was included in the assay. After incubation for 30 h at 20 °C, which is the optimal temperature for expression of Mat fimbriae (Pouttu et al., 2001), the matA(A536C) mutant cells exhibited a minor increase in colony size compared with the parental strain IHE 3034-Rif. In contrast, complementation of the matA substitution with plasmids carrying constitutively expressed matA from the parental IHE 3034 or the commensal K-12 strain MG1655 gave a non-motile phenotype similar to that of the non-flagellated mutant strain IHE 3034-79 (Fig. 1a). A similar inhibitory effect by matAHE 3034 overexpression and a minor positive effect by matA mutation on motility was observed in strain MG1655 (Fig. 1a), which carries the mat operon but is incapable of Mat fimbration expression in vitro (Lehti et al., 2010; Pouttu et al., 2001). Measurement of colony diameters showed that loss of MatA in the IHE 3034 background gave a small and not statistically significant increase in motility at 20 °C but not at 37 °C, whereas overexpression of MatA rendered the bacterium non-motile at 20 °C as well as at 37 °C (Fig. 1b). The motility of IHE 3034 was not significantly affected by the Mat fimbration per se, as an in-frame deletion of the chromosomal matBCDEF structural genes or their complementation in an inducible high-copy-number expression plasmid did not drastically affect motility (Fig. 1a).

To detect whether MatA affects flagellar synthesis, the concentration of flagellin protein was analysed in whole-cell samples by Western blotting using polyclonal anti-FliC_H7 antibodies (Fig. 1c). The amount of FliC was slightly increased in the IHE 3034 matA substitution mutant grown in LB broth at 20 °C compared with that of the parental
strain, whereas the constitutive, ectopic expression of MatA in the mutant strain resulted in undetectable levels of flagellin, similar to the *fliC*-deficient strain. We thus concluded that the regulator MatA, but not Mat fimbria- 

**The fimbriae regulator MatA represses transcription of *flhDC***

To determine whether MatA inhibits flagellar expression at the transcriptional level, the amounts of *flhDC* (controlled by a class 1 promoter) and *fliC* (class 3 promoter) mRNA were analysed by Northern blotting using *flhDC*- and *fliC*-specific probes. The *matA*(*A536C*) mutant strain showed somewhat higher levels of the *flhDC* transcript, approximately 1.1 kb in length, than the wild-type strain at 20 °C as well as at 37 °C (Fig. 2a). When *matA* was expressed either from a constitutive *ptet* promoter in the pACYC184-based plasmid pTAL1 or from an IPTG-inducible *ptrc* promoter in the pSE380-based plasmid pMAT19, the transcription of *flhDC* in the *matA* mutant strain was severely impaired. The *fliC* mRNA, 2.1 kb in length, was detected only in the absence of excess MatA (Fig. 2b).

*flhDC* regulation involves both transcriptional and post-transcriptional control (Wei *et al.*, 2001). To evaluate whether the observed variations in *flhDC* transcript levels could be assigned to transcriptional initiation from the *pflhD* regulatory region, we integrated a *pflhD–lacZ* transcriptional fusion in the chromosome of the wild-type strain and of the isogenic *matA*(*A536C*) mutant. After exponential growth in LB broth at 20 and 37 °C, fusion-directed β-galactosidase activities were monitored. For comparison, expression of a chromosomally encoded *pflC–lacZ* fusion was also analysed in the two strains. The activity of *pflhD* and *pflC* promoters was higher at 20 than at 37 °C (Fig. 3). The absence of functional MatA increased the transcriptional activity of *pflhD* at both temperatures only moderately (Fig. 3a), with a concomitant upregulation (*P*<0.05) of *fliC* transcription (Fig. 3b). We then complemented in trans the *matA* mutation with the pTAL1 plasmid using the pACYC184 vector alone as a control. When *matA* was supplied in trans, the activity of the *flhD–lacZ* fusion was reduced by a factor of 14 at 20 °C and by a factor of eight at 37 °C (Fig. 3a), and showed negligible activity close to the baseline level of the parental strain overexpressing *matA* (<12 Miller units; data not shown). A similar pattern was observed with the *fliC–lacZ* fusion (Fig. 3b). Upon *matA* overexpression, the high *pflC* promoter activity was completely lost at both 20 and 37 °C.

**Binding of MatA to the *flhD* promoter**

Northern blotting and promoter expression analysis demonstrated that MatA influences flagellar biosynthesis by acting as a transcriptional repressor of *flhDC*. To determine whether MatA affects *flhDC* expression by a specific interaction with the *flhD* regulatory region, binding of the MBP–MatA fusion protein to two DNA fragments...
and others, unpublished results). MBP–MatA bound in a concentration-dependent manner to both pflhD fragments, with moderately higher affinity to pflhD1, which contained the immediate upstream region of the flhDC operon (−305 to +28 bp from ATG of flhD, Fig. 4a, b). The binding was of the same affinity as with the pmatA fragment derived from the DNA upstream of matA in strain IHE 3034 (Fig. 4c). MBP–MatA bound more weakly to pfla as well as pfliC (Fig. 4b), and at levels equal to those observed for the binding of MatA to the negative control fragment pmatB harbouring the matA–matB intergenic region (Fig. 4c). No mobility shifting was seen with the purified MBP (Fig. 4).

**DISCUSSION**

Previous work in our laboratory has shown that MatA is a key regulator of the mat operon and is responsible for counteraction of H-NS-mediated repression of the mat locus (T. A. Lehti and others, unpublished results). The mat promoter upstream of the matA gene is positively autoregulated by MatA in the meningitis isolate IHE 3034, and this results in transcription of a polycistronic message that covers matA and the matBCDEF genes, encoding structural components of the Mat fimbriae. The Mat fimbria promotes biofilm formation by IHE 3034 (Lehti et al., 2010), and the Mat/ECP fimbria also mediates bacterial adherence to cultured epithelial cells (Avelino et al., 2010; Blackburn et al., 2009; Lasaro et al., 2009; Rendón et al., 2007; Saldaña et al., 2009). In this study, we provide compelling evidence that, in addition to a role in Mat fimbriae expression, the fimbriae activator MatA decreases expression of the flagellar master operon flhDC, and consequently prevents flagellum biosynthesis and motility, and thereby also taxis. This coordinated switch between motile and sessile lifestyles could have evolved to facilitate a motile-to-adhesive transition in E. coli.

The peritrichously arranged flagella of E. coli provide a highly efficient machinery for directional locomotion, and there are reports that show repression of their expression in adhesive cells. Flagellar synthesis is decreased in cells attached to plastic surfaces as compared with free-living bacteria (Prigent-Combaret et al., 1999) as well as during microcolony formation on epithelial cells (Cleary et al., 2004). Constitutive expression of type 1 fimbriae, an essential virulence factor in UTIs (Connell et al., 2009), has been shown to decrease motility of UPEC (Bryan et al., 2006; Lane et al., 2007a), partially due to the abundance of fimbrial structures at the cell surface (Lane et al., 2007a). Subsequent screening of factors that participate in a decreased-motility phenotype identified four transcriptional regulators, LrhA, Lrp, SlyA and P fimbriae protein PapX, that partially restore motility without affecting type 1 fimbriae expression (Simms & Mobley, 2008a). PapX binds to a 29 bp DNA region located 410 bp upstream of the flhD start codon, and when over-expressed, PapX decreases motility of the UPEC strain CFT073 by repressing flhDC transcription. Interestingly,
papX overexpression had no effect on the motility of the commensal K-12 strain MG1655, despite the presence of an intact binding site for PapX in the flhD promoter (Reiss & Mobley, 2011; Simms & Mobley, 2008b). PapX has the functional homologues MrpJ in Proteus mirabilis (Li et al., 2001; Pearson & Mobley, 2008) and SfaX in NMEC strains (Sjöström et al., 2009a), ectopic overexpression of which represses flagellin production. PapX, SfaX and MprJ do not significantly regulate expression of the corresponding fimbriae (Li et al., 2001; Simms & Mobley, 2008b; Sjöström et al., 2009a, b), but SfaX downregulates type 1 fimbriae expression (Sjöström et al., 2009a). The effect of MatA differs markedly from these transcriptional regulators, as its regulatory activity is required for expression of Mat fimbriae (T. A. Lehti and others, unpublished results), and overexpression of matA does not affect type 1 fimbriation (T. A. Lehti, unpublished results) but represses flagellar synthesis in IHE 3034. Notably, in the K-12 strain MG1655 also, the matA(A536C) substitution slightly increases and matA overexpression totally abolishes motility, and the MatAMG1655 Variant blocks motility, as with MatAHIE 3034. Thus, MatA differs from PapX with respect to the DNA region upstream of flhDC that is recognized by the regulator and in strain specificity. MatA is an example of a novel master fimbriae regulator that also directly interferes with bacterial motility in meningitic as well as K-12 strains of E. coli.

The expression of matA is controlled by the mat promoter, which has significantly higher activity at 20 than at 37 °C in LB (T. A. Lehti and others, unpublished results), and hence presumably also the level of MatA should be higher at low temperature. This assumption is supported by the temperature-dependent Mat fimbrial phenotype of IHE 3034 (Lehti et al., 2010; Pouttu et al., 2001). One would expect that the promoter activity of flhDC and the amount of flhDC mRNA in IHE 3034 would be lower at 20 than at 37 °C due to the repressive action of MatA. Indeed, accumulation of flhDC transcript was more efficient at 37 °C. Surprisingly, the opposite was observed for flhDC promoter activity at the two temperatures. The reasons for this may be the involvement of additional regulatory DNA regions that were not tested here, and the action of CsrA or other regulatory proteins that affect post-transcriptional
regulation of \(\text{flhDC}\) at various temperatures. Moreover, Mat fimbriae are produced in a phase-variable manner (Lehti et al., 2010; Pouttu et al., 2001). In LB broth, only a small subpopulation of cells, up to one-tenth, express Mat fimbriae at 20°C. The phenotypic heterogeneity might mask MatA–\(\text{flhDC}\) cross talk in the IHE 3034 wild-type population. The motility, Northern blotting and reporter experiments in this study evaluated general responses in the cell population. The influence of MatA repression presumably remains low at a population level, and may be the reason for the consistent but minor phenotypic effect of matA mutation on the flagellar gene expression and motility of IHE 3034 observed with all the analyses performed.

\(E.\ coli\) has the ability to thrive in a diverse range of niches. Outside the host, a free-swimming phase allows the bacterium to actively search for more favourable conditions and enables the cell to come into close proximity with inert surfaces, which facilitates the initiation of biofilm formation, an integral protective growth mode (Karatan & Watnick, 2009). Surface binding during type 1 fimbriae-dependent biofilm formation in \(E.\ coli\) K-12 at room temperature is promoted by flagella-driven motility (Pratt & Kolter, 1998), whereas Mat fimbriae support biofilm formation by IHE 3034 at 20°C in both the presence and the absence of flagella (Lehti et al., 2010). Curli adhesin of \(E.\ coli\) mediates biofilm formation independently of motility (Prigent-Combaret et al., 2000). Moreover, the master curli regulator CsgA also inhibits motility, although using mechanisms different from MatA, by binding to the spacer region between two class 2 flagellar operons, \(\text{fliE}\) and \(\text{fliFGHIJK}\) (Ogasawara et al., 2011), and by increasing the synthesis of cyclic-di-GMP by diguanylate cyclase AdrA (Brombacher et al., 2006; Römling et al., 2000). In the host, repression of the flagella system could be beneficial for the bacterium once it has reached the infection site. Notably, during epithelial cell adhesion of enteropathogenic \(E.\ coli\) strain E2348/69, the number of flagellate bacteria is greatly decreased (Cleary et al., 2004), while another study demonstrated that the interaction of E2348/69 with epithelial cells results in increased production of Mat fimbriae (Saldanha et al., 2009). The loss of flagella and the increase in adhesive fimbriae indicate opposite regulation between flagella and Mat fimbriae in the course of host colonization. Based on our results, MatA is likely to be involved in this process. Restriction of flagella expression through MatA may provide a potential mechanism for the maintenance of the delicate balance between intestinal colonization of the bacterium and triggering of host inflammatory responses. The FlhDC regulon also controls several non-flagellar genes, such as genes of metabolic pathways (Fabich et al., 2011; Prüß et al., 2001, 2003; Zhao et al., 2007). Loss of FlhDC has been shown to enhance the metabolic efficiency of the K-12 strain MG1655 by elevating the expression of catabolic genes, which provides a colonization advantage in the intestinal environment (Fabich et al., 2011).

Dynamic regulation of the flagellar system in a reversible manner rather than permanent shut-down of \(\text{flhDC}\) expression by detrimental mutations provides flexibility and allows the bacterium to oscillate between a motile and a sessile state. Our previous results and this report demonstrate that MatA exerts a dual regulatory function on the choice of a planktonic/sessile lifestyle, and further support the existence of an antagonistic control of attachment and motility in \(E.\ coli\). Our ongoing work indicates that MatA is tightly integrated into two separate regulatory circuits, H-NS-MatA–\(\text{flhDC}\) and RcsB-MatA–\(\text{flhDC}\), both of which control expression of the flagellum (T. A. Lehti and others, unpublished results; Lehti et al., 2012).

The ability to selectively repress \(\text{flhDC}\) may increase bacterial fitness during colonization and increase persistence in the environment. Repression of flagella and motility by a fimbriae regulator protein is likely to increase sessile growth of \(E.\ coli\) once the bacterium has reached sites favourable for colonization.

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