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 (Cro xen & 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,

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Abbreviations: ECP, *E. coli* common pilus; EMSA, electrophoretic mobility shift assay; Mat, meningitis-associated and temperature-regulated; MBP, maltose-binding protein; NMEC, newborn meningitis *Escherichia coli*; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.
including P and type 1 fimbriae, which potentiate 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 (Parhasarathy et al., 2007). Similarly, flagella of diarrhoeagenic E. coli have been demonstrated to be directly involved in the adherence to epithelial cells (Giron et al., 2002; Mahajan et al., 2009; Roy et al., 2009) and mucins (Erden 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 (Brown et al., 2009). The transcriptional hierarchy of the flagellar regulon consists of three promoter classes. At the top of the cascade, under the class 1 promoter, is the flhDC operon, which encodes the heteromultimeric transcriptional master activator FlhD4C2 (Komeda, 1986). The master activator controls, together with σ70, the class 2 promoters upstream of the genes encoding the hook–basal body, the flagellar sigma factor FlmA (σ28) and anti-sigma factor FlgM (Kutsukake et al., 1990; Liu & Matsumura, 1994). FlmA is required for the transcription of the class 3 flagellar genes that function at the distal end of the biosynthesis cascade; these include the chemotaxis genes and the flagellin gene (flitC), which encodes the primary structural component FlIC of the flagellum (Ohnishi et al., 1990).

The master regulator FlhD4C2 is required for expression of the other genes of the flagellar regulon and thereby dictates when flagella are produced. Accordingly, initiation of flhDC transcription constitutes the primary regulatory point of the flagellation phenotype, and the flhDC promoter responds to multiple regulatory proteins and a number of environmental as well as physiological factors, such as temperature (Adler & Templeton, 1967; Li et al., 1993), pH (Soutourina et al., 2002) and the presence of butyrate (Tober et al., 2011). Examples of global regulators that directly target the class 1 promoter are the nucleoid protein H-NS (Bertin et al., 1994; Soutourina et al., 1999), the catabolite repressor CRP (Silverman & Simon, 1974; Soutourina et al., 1999), the response regulator RcsB with its accessory cofactor RcsA (Francez-Charlot et al., 2003) and the quorum-sensing regulators QseBC (Clarke & Sperandio, 2005; Sperandio et al., 2002). Moreover, accumulation of the flhDC message is further controlled at the level of mRNA stability by the small RNA-binding protein CsrA (Wei et al., 2001).

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; Saldana 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 fimbriae 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 fimbriae 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 (Rif), 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 pSE380-based plasmid pMAT19 or pMAT6, respectively, was done with 5 μM IPTG.
**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>
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<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</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>
<td>Lehti et al. (2010)</td>
</tr>
<tr>
<td>IHE 3034-79</td>
<td>IHE 3034-Sm fimA::cat siaA::Gm fliC::sat</td>
<td>Pouttu et al. (2001)</td>
</tr>
<tr>
<td>IHE 3034-130</td>
<td>IHE 3034-Rif rrnB T14-pfliD-lacZ (−942 to +22 from the ATG of fliD)</td>
<td>This study</td>
</tr>
<tr>
<td>IHE 3034-131</td>
<td>IHE 3034-Rif rrnB T14-pfliC-lacZ (−712 to +38 from the ATG of fliC)</td>
<td>This study</td>
</tr>
<tr>
<td>IHE 3034-132</td>
<td>IHE 3034-102 rrnB T14-pfliD-lacZ (−942 to +22 from the ATG of fliD)</td>
<td>This study</td>
</tr>
<tr>
<td>IHE 3034-133</td>
<td>IHE 3034-102 rrnB T14-pfliC-lacZ (−712 to +38 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</td>
</tr>
<tr>
<td>MG1655-102</td>
<td>MG1655-Rif matA(A536C), resulting in the substitution MatA H179P</td>
<td>T. A. Lehti</td>
</tr>
<tr>
<td>S17-1::pir</td>
<td>recA thi pro lacRI 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 DE3</td>
<td>Studier &amp; Moffatt (1986)</td>
</tr>
</tbody>
</table>

| **Plasmids** | | |
| pACYC184 | Cloning vector, TcR, CmR | New England Biolabs |
| pTAL1 | IHE 3034 matA under p tet in pACYC184 | T. A. Lehti |
| pTAL2 | MG1655 matA under p tet in pACYC184 | M. Kukkonen |
| pSE380 | Expression vector, trc promoter, ApR | Invitrogen |
| pMAT6 | IHE 3034 matBCDEF under p trc in pSE380 | Pouttu et al. (2001) |
| pMAT19 | IHE 3034 matA under p trc in pSE380 | T. A. Lehti |
| pUC19-placZ | IHE 3034 lacI-pRS551 rrnB T14-partial lacZ in pUC19 | T. A. Lehti |
| pBAU1 | IHE 3034 matA in pMAL-c2x | P. Bauchart |

**Construction of single-copy lacZ reporter strains.** Recombinant DNA techniques were carried out using standard protocols (Sambrook & Russell, 2001). The *pfliD* 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 *pfliD* (−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 BanHI restriction site of plasmid pUC19-placZ, which contains the IHE 3034 *lacI* gene and its upstream region, as well as four *rrnB* T1 terminators, a promoter cloning site and a part of the promoterless *lacZ* gene from reporter plasmid pRS551. The resulting plasmids were digested with XbaI and SacI, and the restriction fragments containing the *pfliD* 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 *pfliD* or *pfliC* promoter on the chromosome of IHE 3034-Rif or its *matA(A536C)*

**Table 2. Primers used in this study**

<table>
<thead>
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<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Purpose</th>
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<td>315pfliD-942F</td>
<td>CGCGGATCCCAACGCATGGCGCGACAGTAAT</td>
<td><em>pfliD</em>–<em>lacZ</em> fusion; BanHI</td>
</tr>
<tr>
<td>241pfliD222R</td>
<td>CGCGGATCCCTGGAACACTCGGAGGTATGC</td>
<td><em>pfliD</em>–<em>lacZ</em> fusion; BanHI</td>
</tr>
<tr>
<td>316pfliC-712F</td>
<td>CGCGGATCCGACGATTTGACCCGCGTGTAT</td>
<td><em>pfliC</em>–<em>lacZ</em> fusion; BanHI</td>
</tr>
<tr>
<td>243pfliC388R</td>
<td>CGCGGATCCATCGAGAGAGGTGTAT</td>
<td>Screening of promoter–<em>lacZ</em> fusions</td>
</tr>
<tr>
<td>191lacI</td>
<td>TTAATGCAGCAGCCAGCACG</td>
<td>Screening of promoter–<em>lacZ</em> fusions</td>
</tr>
<tr>
<td>035lacZ</td>
<td>GTCGTTATGCGACGAC</td>
<td>Sequencing of promoter–<em>lacZ</em> fusions</td>
</tr>
<tr>
<td>190pRS551R</td>
<td>GGCCACTGGAATCCGTAATC</td>
<td><em>pfliD1</em> EMSA fragment</td>
</tr>
<tr>
<td>146fliD1F</td>
<td>CATTTATGTTAAGTAATGAG</td>
<td><em>pfliD1</em> EMSA fragment</td>
</tr>
<tr>
<td>148fliD1R</td>
<td>TGTTTTGACAGATCCTG</td>
<td><em>pfliD1</em> EMSA fragment</td>
</tr>
<tr>
<td>145fliD2F</td>
<td>CGCGGATCCATCGAGAGAGGTGTAT</td>
<td><em>pfliD2</em> EMSA fragment</td>
</tr>
<tr>
<td>147fliD2R</td>
<td>CTCAATTCATTAAATGATG</td>
<td><em>pfliD2</em> EMSA fragment</td>
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<tr>
<td>234pfliCF</td>
<td>CTGCGGATCCATCGAGAGAGGTGTAT</td>
<td><em>pfliC</em> EMSA fragment</td>
</tr>
<tr>
<td>235pfliCR</td>
<td>GACTTGGCCCAGATTG</td>
<td><em>pfliC</em> EMSA fragment</td>
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<tr>
<td>236pfliAF</td>
<td>AGCAACGTGTTCCG</td>
<td><em>pfliA</em> EMSA fragment</td>
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<tr>
<td>237pfliAR</td>
<td>GATGAAATCAGATAAAC</td>
<td><em>pfliA</em> EMSA fragment</td>
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</table>
Repression of fliDC by an E. coli fimbriae activator

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.

For quantification of fliDC and fltC 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 fliDC or fltC genes were amplified from chromosomal DNA of E. coli IHE 3034 and labelled with PCR DIG Labelling 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-laurylsarcosine, 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 (maltoolose-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 amylose 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 Isotopenmessgeräte GmbH).

Electrophoretic mobility shift assays (EMSSAs). DNA fragments for binding assays, pfliD1 (−305 to +28 from the ATG of fliD), pfliD2 (−1009 to −285 from the ATG of fliD), pfliC (−273 to +12 from the ATG of fliC), pfliA (−158 to +12 from the GTG of fliA), pmatA (−343 to +10 bp from the GTG of matA) and pmatB (−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 % (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.

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, 2 μl of cultures grown overnight at 20 or 37 °C in LB broth was carefully spotted in the centre of the plates, and incubated for 30 h at 20 °C or for 7.5 h at 37 °C before the diameter of the colony was measured for each strain. The motility phenotype of LB broth-cultivated bacteria was confirmed by phase-contrast microscopy using an Olympus CKX41 microscope.

Detection of FliC 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, transferred onto a nitrocellulose membrane and detected with anti-H7 flagella antibodies (1 : 5000), followed by alkaline phosphatase-conjugated swine anti-rabbit immunoglobulins (1 : 10000, Dako) and phosphatase substrate. The samples were also 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 heparin (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 fliDC and fltC 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 fliDC or fltC genes were amplified from chromosomal DNA of E. coli IHE 3034 and labelled with PCR DIG Labelling 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-laurylsarcosine, 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).

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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 (Pouuttu 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 communal 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 matA<sub>IHE 3034</sub> 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 fimbriation expression <em>in vitro</em> (Lehti et al., 2010; Pouuttu 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 fimbriation 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<sub>H7</sub> 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

**Fig. 1.** 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 <em>ptet</em> promoter of the vector. The non-flagellated <em>flic</em>::<em>sat</em> 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.
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 fimbriae as such, affects motility in E. coli by repressing fliC expression.

**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 pfhlD regulatory region, we integrated a pfhlD–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 pfliC–lacZ fusion was also analysed in the two strains. The activity of pfhlD and pfliC promoters was higher at 20 than at 37 °C (Fig. 3). The absence of functional MatA increased the transcriptional activity of pfhlD 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 pfliC 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...
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 result in transcription of a polycistronic message that covers matA and the matBCDEF genes, encoding structural components of the Mat fimbriae. The Mat fimbia promotes biofilm formation by IHE 3034 (Lehti et al., 2010), and the Mat/ECP fimbia 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 fimbiae expression, the fimbiae 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., 2004), 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 fimbiae expression (Simms & Mobley, 2008a). PapX binds to a 29 bp DNA region located 410 bp upstream of the flhD start codon, and when overexpressed, PapX decreases motility of the UPEC strain CFT073 by repressing flhDC transcription. Interestingly, 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 pflhA as well as pflhC (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).

**Fig. 3.** Downregulation of flhD and flhC promoter activities by MatA. β-Galactosidase activities expressed from the wild-type IHE 3034 Rif strain and matA derivatives harbouring the single-copy pflhD–lacZ (a) and pflhC–lacZ (b) transcriptional fusions at the chromosomal lac locus. The effect of ectopic expression of matA on the promoter activities was tested by introducing a pACYC184-based matA expression plasmid into the matA(A536C) reporter strains. The analyses were performed using exponential-phase cultures grown in LB broth at 20 or 37 °C. Error bars, SD of three independent assays. One asterisk, P<0.05; two asterisks, P<0.005, as calculated by Student’s t test.

(pflhD1 and pflhD2) originating from the flhD promoter was analysed by EMSA. For comparison, flagellar class 2 (pflhA) and class 3 (pflhC) as well as matA and matB promoter fragments were also analysed. We have previously observed that MatA binds to the pmatA fragment but not efficiently to the pmatB fragment (T. A. Lehti...
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 flagelin production. PapX, SfaX and MprJ do not significantly regulate expression of the corresponding fimbrae (Li et al., 2001; Simms & Mobley, 2008b; Sjöström et al., 2009a, b), but SfaX downregulates type 1 fimbrae 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 fimbrae (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 MatA_IHE 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 fimbrae 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 \( flhDC \) 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–\( flhD \) 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 adhesion of \( E. \ coli \) mediates biofilm formation independently of motility (Prignet-Combet 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, flIE and flITFGHIJK (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 (Saldaná 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 \( flhDC \) 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-\( flhDC \) and RcsB-Mata-\( flhDC \), both of which control expression of the flagellum (T. A. Lehti and others, unpublished results; Lehti et al., 2012).

The ability to selectively repress \( flhDC \) 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.

ACKNOWLEDGEMENTS

We thank Maini Kukkonen, Supervisory Authority for Welfare and Health, Helsinki, Finland, for providing plasmid pTAL2. We would also like to thank Raili Lameranta and Elina Nummenmaa for technical assistance. This work was financially supported by University of Helsinki, Viikki Doctoral Programme in Molecular Biosciences, Academy of Finland (in the frame of the ERA-NET PathoGenoMics grants 118982 and 130202, and General research grant 123900), and the German Ministry of Education and Research (ERA-NET PathoGenoMics grant 0313937A). U. D. was supported by the German Research Foundation (grant number DO 789/4-1), and P. B. received a PhD fellowship of the Bavarian Research Foundation. This work was carried out in the frame of the European Virtual Institute for Functional Genomics of Bacterial Pathogens (CEE LSHB-CT-2005-512061).

REFERENCES


Repression of \textit{flhDC} by an \textit{E. coli} fimbriae activator


Edited by: A. H. M. van Vliet