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YjjQ Represses Transcription of *flhDC* and Additional Loci in *Escherichia coli*

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ABSTRACT

The presumptive transcriptional regulator YjjQ has been identified as being virulence associated in avian pathogenic *Escherichia coli* (APEC). In this work, we characterize YjjQ as transcriptional repressor of the *flhDC* operon, encoding the master regulator of flagellar synthesis, and of additional loci. The latter include *gfc* (capsule 4 synthesis), *ompC* (outer membrane porin C), *yfiRNB* (regulated c-di-GMP synthesis), and loci of poorly defined function (*ybhL* and *ymiA-yciX*). We identify the YjjQ DNA-binding sites at the *flhDC* and *gfc* promoters and characterize a DNA-binding sequence motif present at all promoters found to be repressed by YjjQ. At the *flhDC* promoter, the YjjQ DNA-binding site overlaps the RcsA-RcsB DNA-binding site. RcsA-RcsB likewise represses the *flhDC* promoter, but the repression by YjjQ and that by RcsA-RcsB are independent of each other. These data suggest that YjjQ is an additional regulator involved in the complex control of *flhDC* at the level of transcription initiation. Furthermore, we show that YjjQ represses motility of the *E. coli* K-12 laboratory strain and of uropathogenic *E. coli* (UPEC) strains CFT073 and 536. Regulation of *flhDC*, *yfiRNB*, and additional loci by YjjQ may be features relevant for pathogenicity.

IMPORTANCE

***Escherichia coli* is a commensal and pathogenic bacterium causing intra- and extraintestinal infections in humans and farm animals. The pathogenicity of *E. coli* strains is determined by their particular genome content, which includes essential and associated virulence factors that control the cellular physiology in the host environment. However, the gene pools of commensal and pathogenic *E. coli* are not clearly differentiated, and the function of virulence-associated loci needs to be characterized. In this study, we characterize the function of *yjjQ*, encoding a transcription regulator that was identified as being virulence associated in avian pathogenic *E. coli* (APEC). We characterize YjjQ as transcriptional repressor of flagellar motility and of additional loci related to pathogenicity.**

Escherichia coli is a tremendously well-characterized genetic model organism and an invaluable tool in genetic engineering. However, *E. coli* is also an important pathogen causing a variety of intra- and extraintestinal infections in vertebrates, including humans and farm animals. The specific pathotype of individual *E. coli* strains is presumptively determined by their particular genome, which is composed of core and variable gene loci, including horizontally acquired genes (1, 2). The variable gene pool includes essential virulence factors and loci that apparently affect and regulate the cellular physiology and metabolism in the host environment (1). For example, the B2 lineage of the four phylogenetic lineages of *E. coli* A, B1, B2, and D (3, 4) includes a high proportion of extraintestinal and avian pathogenic strains (1, 4). However, currently the gene pools of commensal and various pathogenic *E. coli* strains are not clearly differentiated, and it is necessary to further characterize specific virulence traits (1, 5).

One of the many loci that have been identified as being virulence associated is *yjjQ*, encoding a putative transcription regulator (6, 7). This locus was found in a signature-tagged transposon mutagenesis screen of avian pathogenic *E. coli* (APEC) strain IMT5155 (O2:H5:K1) using a chicken infection model (7). Analyses of the Tn*5* transposon-tagged APEC IMT5155 *yjjQ* mutant indicated a downregulation of iron metabolism-related genes (6). Furthermore, a Tn*5* transposon-tagged *yjjQ* mutant was identified in a screen for mutants of uropathogenic *E. coli* (UPEC) strain CFT073 that are motile when simultaneously expressing type I fimbria. However, this phenotype was not confirmed with an isogenic *yjjQ* deletion mutant (8).

The *yjjQ* gene is the first gene of the *yjjQ-bglJ* operon. The second gene, *bglJ*, also encodes a transcriptional regulator that has a pleiotropic function (9–11). Transcription of the *yjjQ-bglJ* operon is repressed by the global repressor H-NS (heat-stable nucleoid structuring protein), and it is activated by the pleiotropic transcriptional regulator LeuO (12). The physiological conditions that cause induction of the *yjjQ-bglJ* operon are unknown, which resembles the current lack of knowledge about H-NS-repressed and LeuO-activated loci (13–17). However, regulation of the *yjjQ-bglJ* operon by H-NS and LeuO indicates that these genes are

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related to the control of stress responses and/or virulence-associated traits (15, 18, 19).

In this work, we addressed the function of the YjjQ protein. YjjQ is a dimeric presumptive transcriptional regulator carrying an FixJ/NarL-type C-terminal helix-turn-helix DNA-binding domain (11). We identify gene loci regulated by YjjQ and address the putative regulation of their promoters. We characterize a YjjQ DNA-binding motif and specific DNA-binding sites by mutagenesis. Furthermore, we show that YjjQ specifically represses the promoter of the *flhDC* operon encoding the master regulator $FlhD_4C_2$ of flagellar synthesis (20), among other loci. Correspondingly, ectopically expressed YjjQ inhibits motility of the *E. coli* laboratory strain K-12 as well as that of human extraintestinal pathogenic *E. coli* strains, such as UPEC strains CFT073 and 536.

MATERIALS AND METHODS

Strains, plasmids, and oligonucleotides. The strains, plasmids, and oligonucleotides used in this study are listed in Tables S1, S2, and S3 in the supplemental material. Bacteria were grown in LB medium (10 g tryptone, 5 g yeast extract, and 5 g NaCl per liter). Antibiotics were added to final concentrations of 25 μg/ml kanamycin, 15 μg/ml chloramphenicol, 50 μg/ml spectinomycin, and 50 μg/ml ampicillin where indicated. Plasmid cloning followed standard techniques (21). For mutagenesis of the *flhDC* promoter fragment, the PCR was performed with an unbalanced nucleotide ratio, as described previously (22).

Motility and biofilm assay. For motility assays, the centers of LB soft agar plates (0.25% [wt/vol] Bacto agar supplemented with 25 μg/ml kanamycin, and 200 μM isopropyl-β-D-thiogalactopyranoside [IPTG] where indicated) were spotted with 3 μl of an overnight culture. Swimming radii were determined after incubation for 5 h at 37°C for K-12 strain BW30270 and after 12 h at 28°C for the UPEC strains. Biofilm assays were performed as described previously (57). Briefly, overnight cultures were diluted 100-fold in LB kanamycin medium supplemented with 1 mM IPTG where indicated. Of these dilutions, 100 μl was grown for 48 h at 28°C in quadruplets in 96-well microtiter plates with lids (flat bottom, polystyrene, non-tissue culture treated). The bacterial suspension was removed by pipetting, and the wells were washed once with 150 μl H_2O. Then 150 μl 0.1% (wt/vol) crystal violet was added to each well, and the plates were incubated for 10 min at room temperature. The dye was removed by pipetting, the wells were washed twice with 200 μl H_2O, and the plates were air dried. To solubilize the dye, 200 μl of 30% acetic acid was added to each well, and the plates were incubated at room temperature for a few minutes.

Mapping of transcription start sites by 5′-RACE. Mapping of the transcription start sites by 5′ rapid amplification of cDNA ends (5′-RACE) was performed as described previously (10, 23). Briefly, RNA was isolated from strain T23 [Δ(*yjjP-yjjQ-bglJ*)] grown in LB to an optical density at 600 nm (OD_{600}) of 0.5 using the RNAprotect and RNeasy minikit system with on-column DNase digestion (Qiagen, Germany). To distinguish primary 5′ ends of transcripts and 5′ ends generated by processing, 6 μg of the RNA was treated with tobacco acid pyrophosphatase (TAP) (Epicentre Biotechnologies). Then an RNA adapter (RNA oligonucleotide T268) was ligated to both 6 μg of untreated RNA and to the TAP-treated RNA preparations. Following phenol-chloroform-isoamyl alcohol (25:24:1) extraction and ethanol precipitation, the RNA samples were used for first-strand cDNA synthesis with random hexameric oligonucleotides as primers and the SuperScript III First Strand synthesis kit according to the instructions of the manufacturer (Invitrogen, Germany). The cDNA was used for PCRs with adapter-specific primer T265 and gene-specific primers (see Table S3 in the supplemental material). TAP-specific PCR fragments obtained for each locus were digested with EcoRI and XbaI, and the fragments were cloned into pUC12. At least 3 clones were sequenced for mapping of the primary transcription start sites.

TABLE 1 Putative YjjQ-regulated loci in *E. coli* K-12 and UPEC CFT073 identified by microarray analysis

Locus	Fold repression[a]		Function
	K-12	CFT073	
adiY	39.6	29.3	Arginine-dependent acid resistance
cbpA	25.3	8.0	DNA-binding cochaperone system
csrB	11.3	5.2	srRNA
flhDC	Mutant[b]	9.8/6.0	Master regulator of flagellar synthesis
gfcA	53.1[c]		Group 4 capsule synthesis
mdtJ	31.1	13.7	Spermidine exporter
nanC	11.5	1.1	Sialic acid metabolism
ompC	36.6	17.2	Porin
panD	11.4	10.4	L-Aspartate-α-decarboxylase
ucpA	22.0	8.7	Furan resistance
uspF	6.4	10.6	Universal stress protein
yfiR	23.4	7.8	Periplamic regulator of c-di-GMP synthase YfiN
yhjR	15.5	12.9	Cellulose synthesis
bcsE	9.0	7.9	Cellulose synthesis
c1618		21.4	Unknown
ybhL	26.8	19.3	Inner membrane transporter
ymiA/yciX	23.3/28.6	10.4/10.1	Unknown

[a] Listed are the fold repression values caused by expression of *yjjQ* carried by plasmid pKERV17 in transformants of *E. coli* K-12 strain S3922 and UPEC strain CFT073. Shown are the repression values for the first gene of each operon. For *flhDC* and *ymiA/yciX*, the folds of repression for both genes of the operon are given.
[b] *E. coli* K-12 strain S3922 [BW30270 Δ(*yjjP-yjjQ-bglJ*)::KD3] is nonmotile, and the *flhDC* promoter region is rearranged.
[c] The *gfc* operon is not expressed in *E. coli* K-12 due to an IS*1* insertion element. However, the microarray probe maps between the promoter and the IS*1* element.

Expression analyses. For expression analyses of *lacZ* reporter fusions, β-galactosidase assays were performed as described previously (10, 24). Briefly, bacterial cultures were inoculated from fresh overnight cultures to an OD_{600} of 0.05 and grown to the exponential-growth-phase OD_{600} of 0.5 in LB medium. The medium of the overnight and exponential cultures was supplemented with antibiotics and 1 mM IPTG where indicated. The average units are based on at least three biological replicates, and in each assay, at least 4 technical replicates were measured.

To determine putative target genes of YjjQ, the Δ(*yjjP-yjjQ-bglJ*) strain S3922 was transformed with *yjjQ*-containing plasmid pKERV17 or the empty vector control pKES169. The transformants were grown in LB medium supplemented with kanamycin to an OD_{600} of 0.3, and then IPTG was added for induction, and the cultures were grown for additional 30 min to an OD_{600} of approximately 0.5. RNA was isolated using the RNAprotect and RNeasy minikit system with on-column DNase digestion (Qiagen, Germany). Hybridization to Affymetrix GeneChip *E. coli* Genome 2.0 microarrays was carried out according to the manufacturer's instructions. Microarrays were scanned using an Affymetrix GeneChip Scanner 3000 7G. Data were processed using the Affymetrix apt-probeset-summarize software (version 1.10) and RMA algorithm. Samples were normalized using the standard normalization probes present on the Affymetrix GeneChip, and differential expression values were calculated as fold changes. Data for all loci for which a 10-fold or higher repression in at least one of the strains was detected are shown in Table 1.

RESULTS

Regulation of presumptive target promoters by YjjQ. To identify target genes of the putative transcriptional regulator YjjQ, we analyzed the change of the expression pattern caused by plasmidic expression of *yjjQ* using a microarray approach. In this assay, high-level expression of *yjjQ* that was provided by high-copy-number plasmid pKERV17 caused downregulation of approxi-

mately 20 loci, both in the *E. coli* K-12 *yjjQ* deletion strain S3922, and in the UPEC strain CFT073 (Table 1). The UPEC strain CFT073 was chosen because UPEC and APEC strains are closely related (25, 26) and a representative microarray was available for CFT073 at that time but not for APEC strain IMT5155, in which *yjjQ* was identified as being virulence associated. To validate whether the presumptive target loci identified by this microarray analysis are indeed regulated by YjjQ at the level of transcription initiation, we chose 16 of the loci for construction of promoter *lacZ* fusions (see Fig. S1 in the supplemental material). These promoter-*lacZ* reporter fusions were integrated into the *attB* site of the *E. coli* K-12 *yjjQ* and *lacZ* deletion strain T23 [Δ*lacZ* Δ(*yjjP-yjjQ-bglJ*)] (Fig. 1; see Fig. S1). Regulation of these *lacZ* reporter fusions by YjjQ was tested using the low-copy-number *yjjQ*-expressing plasmid pKEKD31 and an empty vector control (Fig. 1; see Fig. S1). YjjQ was provided plasmidically, as expression of the native *yjjQ-bglJ* operon is repressed by H-NS during exponential growth under laboratory conditions (12). The expression analyses of the promoter-*lacZ* reporter fusions revealed that 6 of the 16 putative target promoters are repressed by YjjQ (Fig. 1), while the activities of the remaining promoters were unaffected under the conditions used (see Fig. S1). Possibly high expression levels of *yjjQ* in the microarray yielded that many false positives. Repression by YjjQ using the promoter-*lacZ* reporters was detected for the promoters of *flhDC*, *ompC*, *gfc*, *ybhL*, *yfiRNB*, and *ymiA-yciX* (Fig. 1). For comparison, these six YjjQ-repressed promoter-*lacZ* fusions were also tested in the *yjjQ*$^+$ wild-type background S4197 (Δ*lacZ*) (Fig. 1), with similar results as in the *yjjQ* deletion strain (Fig. 1), which demonstrated that the presence of wild-type *yjjQ* at its native, H-NS-repressed locus has no effect. Of the six YjjQ repressed loci, *flhDC* encodes the master regulator $FlhD_4C_2$ of flagellar synthesis (27). This locus is repressed most by YjjQ (10-fold). Please note that in the initial microarray analysis, *flhDC* was identified as the putative target in UPEC strain CFT073 but not in the K-12 strain S3922. The latter strain, S3922, turned out to be nonmotile and to carry a mutation at the *flhDC* locus, which may explain this discrepancy. Other targets of YjjQ are *ompC*, encoding the outer membrane porin OmpC (28), and the *gfc* locus, encoding enzymes for group 4 capsule synthesis (29). The *gfc* locus is not expressed in *E. coli* K-12 due to an IS*1* insertion between the promoter and the coding region (29). However, in the *gfc* promoter-*lacZ* reporter, this IS*1* element was excluded. The *ybhL* gene product is an inner membrane protein and putative transporter (28). The *yfiRNB* locus encodes the c-di-GMP cyclase YfiN, the periplasmic regulatory protein YfiR, and the lipoprotein YfiB. Mutation of *yfiR* affects swarming and swimming motility, as well as early biofilm formation (28, 30). Furthermore, the Yfi system promotes CsgD-independent cellulose production under reducing conditions (31). Likewise, in the UPEC strain CFT073, *yfiR* mutants are affected in synthesis of curli and cellulose, and they are attenuated in mouse bladder and kidney infection (32). The YjjQ target locus *ymiA-yciX* codes for the small putative membrane protein YmiA (42 amino acids [aa]) (33) and a small putative protein YciX (55 aa) of unknown function (28).

Mapping of YjjQ-binding sites. YjjQ-mediated repression of the target gene promoters presumably involves DNA binding by YjjQ. To map the YjjQ DNA-binding sites, the DNA sequences encompassing the YjjQ-regulated target promoters from positions −100 to +50 relative to their transcription start sites were searched for a common motif using the program MEME Suite (34). The position of the transcription start sites were taken from the literature for the *flhDC* and *ompC* promoters (for references, see reference 28), while they were mapped by 5′-RACE for the *ybhL*, *ymiA-yciX*, *yfiRNB*, and *gfc* promoters (see Fig. S2 in the supplemental material). The transcription start sites of the non-YjjQ-regulated promoters of the *bcsE*, *yhjR*, and *panD* genes were also mapped by 5′-RACE (see Fig. S1C). Indeed, the MEME Suite

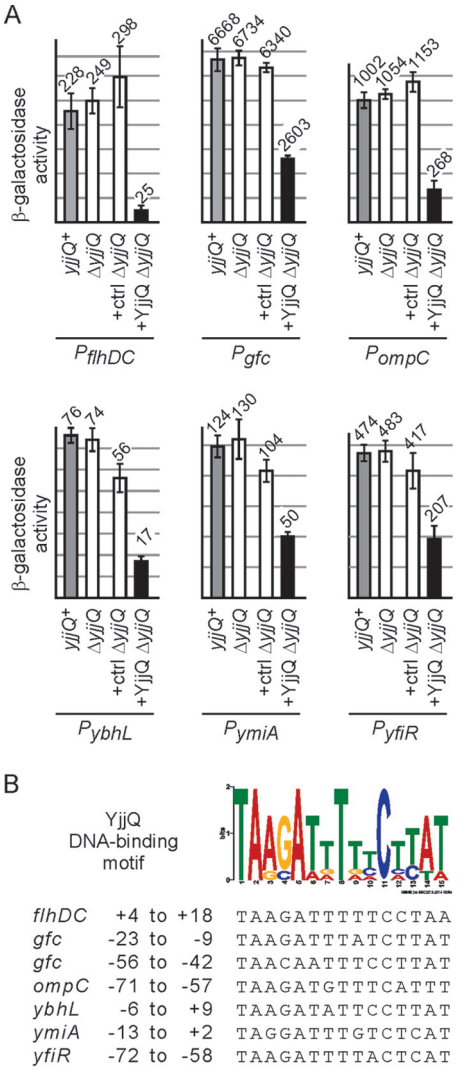

FIG 1 Regulation of target gene promoters by YjjQ and identification of a YjjQ DNA-binding motif. (A) The expression level of chromosomal promoter-*lacZ* reporter fusions was determined in the *yjjQ*$^+$ strain background S4197 as well as in the *yjjP-yjjQ-bglJ* deletion strain T23 (Δ*yjjQ*). Further transformants of T23 (Δ*yjjQ*)-derived reporter strains with vector control plasmid pKESK22 (ctrl) and the *yjjQ*-containing plasmid pKEKD31 (+ YjjQ) were analyzed. Bacteria were grown to the mid-exponential phase (OD_{600} of 0.5) in LB medium, which was supplemented with kanamycin and 1 mM IPTG in the case of transformants. The β-galactosidase activities of at least three biological replicates were determined. The following strains were used: T2047 and T1266 (P_{flhDC}), T2051 and T1541 (P_{gfc}), T2046 and T1268 (P_{ompC}), T2049 and T1533 (P_{ybhL}), T2050 and T1535 (P_{ymiA}), and T2048 and T1351 (P_{yfiR}). (B) Analysis of 150-bp DNA sequence encompassing the target promoters using MEME Suite (34) yielded a putative YjjQ DNA-binding motif. The individual sequences matching this motif in the target promoter fragments and their relative positions to the transcription start site are indicated at the bottom. At the *gfc* promoter, two sites were identified.

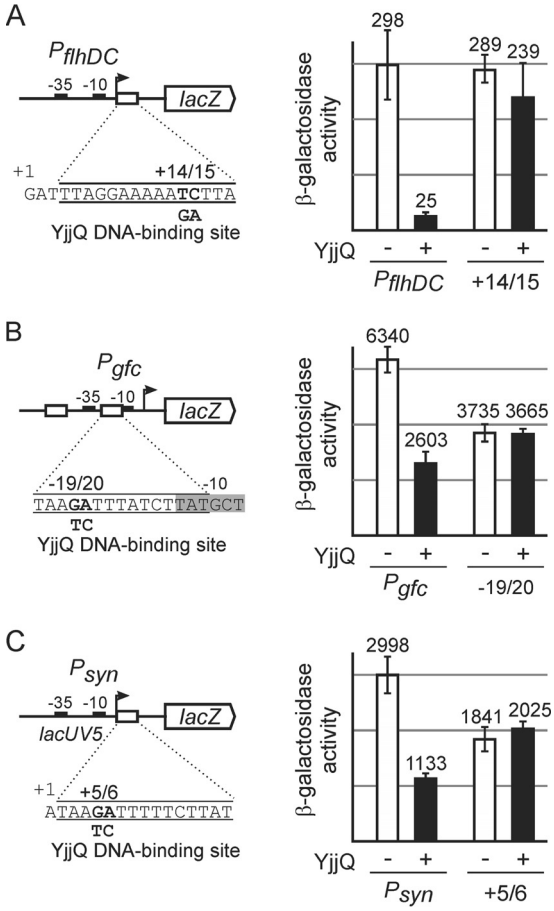

FIG 2 Mutation of the putative YjjQ DNA-binding site abrogates repression by YjjQ. Promoter-*lacZ* fusions and their mutant derivatives carrying mutations in the putative YjjQ DNA-binding site were integrated in strain T23. The expression level was determined for transformants of these reporter strains with control plasmid pKESK22 (−) and *yjjQ*-containing plasmid pKEKD31 in the presence of YjjQ (+). Schematically shown are the main features of the promoter-*lacZ* fusions, such as the transcription start site (+1), the −10 and the −35 boxes, as well as the sequences of the putative YjjQ DNA-binding sites and the mutations that were introduced. Tested were the *flhDC* promoter (P_{flhDC}) (strain T1266) and its mutant derivative (+14/15) carrying a 2-nucleotide exchange at positions +14 and +15 relative to the transcription start (strain T1661) (A), the *gfc* promoter (P_{gfc}) (strain 1541) and its mutant derivative (−19/20 [strain T1664]) (B), and a synthetic promoter consisting of the *lacUV5* core promoter and the YjjQ DNA-binding motif fused immediately downstream of the transcription initiation site (strain T1675), as well as its mutant derivative (+5/6 [strain T1667]) (C). Transformants of these reporter strains with the control plasmid pKESK22 (− YjjQ) and with plasmid pKEKD31 (+ YjjQ) were grown to an OD_{600} of 0.5 in LB medium supplemented with kanamycin and IPTG. Given is the average of enzyme activities determined from at least 3 independent biological replicates.

search of the YjjQ target promoter sequences yielded a motif present in all six fragments (Fig. 1; see Fig. S2). This 15-bp motif is palindromic, as it is typical for a DNA-binding site of a homodimeric transcriptional regulator and is of similar size to other DNA-binding motifs characterized for transcription regulators of the FixJ/NarL family (35, 36). The YjjQ DNA-binding motif overlaps with the core promoter or transcription initiation region in the cases of *flhDC*, *gfc*, *ybhL*, and *ymiA* (Fig. 2; see Fig. S2). At the *gfc* promoter, the motif sequence was identified twice, with one of them mapping between the −35 and −10 promoter sequences (see Fig. S2). At the *ompC* promoter, the motif maps upstream of the core promoter (centered at position −64 relative to the transcription start site of the main P1 promoter) and overlaps the binding sites of the activator OmpR (see Fig. S2). Likewise, at *yfiR* the motif maps upstream of the promoter, with the center of the motif mapping at position −65 relative to the transcription start site (see Fig. S2).

To experimentally validate whether the motif represents the YjjQ DNA-binding site, we chose the *flhDC* and the *gfc* promoters and mutated the motif sequence in the respective promoter-*lacZ* reporter fusions, as shown in Fig. 2. Expression analyses of these mutants in the absence and presence of YjjQ demonstrated that the mutation of the YjjQ DNA-binding motif abrogates repression of these promoters by YjjQ (Fig. 2). In the case of the *flhDC* promoter, the mutation of the YjjQ DNA-binding motif rendered the promoter YjjQ independent and affected the activity of the promoter only marginally (Fig. 2A). In the case of the *gfc* promoter, the mutation of the YjjQ DNA-binding motif likewise abrogated repression by YjjQ (Fig. 2B). However, the mutation also caused an approximately 2-fold reduction of the promoter activity (Fig. 2B). This reduction of activity is likely to be based on the proximity of the mutation to the −10 sequence. In a second approach to validate the YjjQ DNA-binding motif, we fused this motif to the *lacUV5* core promoter (P_{lacUV5}) and analyzed whether this confers repression by YjjQ (Fig. 2C). This synthetic P_{lacUV5} YjjQ motif construct was repressed 3-fold by YjjQ (Fig. 2C). As control, fusion of a mutant YjjQ DNA-binding motif to the *lacUV5* core promoter did not confer repression by YjjQ (Fig. 2C). Again the mutation, which maps next to the transcription initiation site of this synthetic promoter, affected the promoter and reduced its activity approximately 1.5-fold (Fig. 2C). Taken together, the data from the mutational analyses suggest that the motif represents the YjjQ DNA-binding site.

As a further unbiased approach to identify the YjjQ DNA-binding site, we performed a mutagenesis of the *flhDC* promoter fragment and screened for promoter mutants that are YjjQ independent (Fig. 3). For this mutagenesis screen, the *flhDC* promoter fragment was amplified by PCR using error-prone conditions and cloned into the *lacZ* reporter plasmid pKES268. The clones were screened for a changed phenotype in strain T1124 (P_L*yjjQ* Δ*lacZ*), which carries a *yjjQ* allele that is constitutively expressed under the control of the phage lambda P_L promoter. Colonies were screened on tetrazolium chloride lactose indicator plates, and clones with a phenotype different from that of the wild type were picked. This screen yielded 4 independent mutants mapping to 3 different nucleotides within the YjjQ DNA-binding site (Fig. 3). In addition, several clones with mutations mapping within the core promoter were isolated (see Fig. S3 in the supplemental material). The latter turned out to be promoter up- and promoter down-mutations (see Fig. S3). Intriguingly, all 3 mutations mapping within the YjjQ DNA-binding motif abrogated repression of the *flhDC* promoter by YjjQ but caused no change of the promoter activity, as shown by expression analyses (Fig. 3). For these expression analyses, the *flhDC* promoter and its mutants were integrated into the chromosome of strain T1124 carrying the P_L*yjjQ* allele (conferring constitutive *yjjQ* expression) and strain T23 carrying a *yjjQ* deletion (Fig. 3). The data support the conclusion that the motif represents the YjjQ DNA-binding site.

Control of *flhDC* by YjjQ and RcsAB. The putative YjjQ DNA-binding site at the *flhDC* promoter overlaps the DNA-binding site

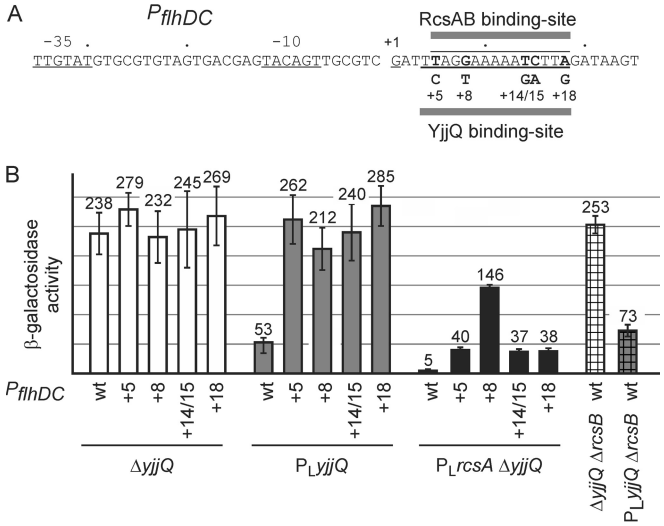

FIG 3 Characterization of YjjQ-independent mutants of the *flhDC* promoter and their regulation by RcsB and RcsA-RcsB. (A) Sequence of the *flhDC* promoter and YjjQ independent mutants mapping within the YjjQ DNA-binding site. The mutations at +5, +8, and +18 were identified in a mutagenesis screen, while mutations at +14 and +15 (+14/15) were generated by site-directed mutagenesis. The YjjQ DNA-binding site overlaps with the RcsAB box that was characterized as the DNA-binding site of RcsA-RcsB (37). (B) The wild-type and mutant *flhDC* promoter-*lacZ* reporter fusions were integrated chromosomally, and their expression was tested in the *yjjQ* deletion strain T23 (Δ*yjjQ*) and in strain T1124 carrying the *yjjQ* P_L*yjjQ* allele that is constitutively expressed under the control of the phage lambda P_L promoter. Furthermore, for constitutive expression of *rcsA* under the control of the lambda P_L promoter, allele P_L*rcsA* was transduced, resulting in P_L*rcsA* Δ*yjjQ* strain backgrounds (see Table S1 in the supplemental material). In addition, Δ*rcsB* deletion mutants that carry a *yjjQ* deletion (Δ*yjjQ*) (strain T1674) or constitutive *yjjQ* allele, P_L*yjjQ* (strain T1771), were used. The reporter strains were grown to the mid-exponential phase (OD_{600} of 0.5) in LB medium, and average values from at least three biological replicates are shown.

for RcsA-RcsB and for RcsB that was identified previously (37) (Fig. 3). Therefore, repression of *flhDC* by YjjQ might be indirect and depend on RcsB or RcsA-RcsB. To address this, we analyzed repression of the *flhDC* promoter by YjjQ in an *rcsB* deletion background. In this strain background, repression can be mediated neither by RcsB nor by the heteromeric RcsA-RcsB. The expression data show that YjjQ represses the *flhDC* promoter-*lacZ* fusion similarly in the Δ*rcsB* background as in the isogenic $rcsB^+$ strain (Fig. 3). This suggests that YjjQ-mediated repression is RcsB as well as RcsA-RcsB independent. Notably, the expression levels are moderately higher in the Δ*rcsB* mutant than in the wild type, which may be attributable to a weak repression of the *flhDC* promoter by the response regulator RcsB, even without induction of the Rcs signaling cascade. This is in accordance with previous data, where it was shown that overexpression of RcsB causes repression of *flhDC* (37). Furthermore, we analyzed RcsA-RcsB-mediated repression of the *flhDC* promoter-*lacZ* fusion and its YjjQ DNA-binding motif mutants. To analyze regulation of *flhDC* by RcsA-RcsB independently of regulation of *rcsA*, we replaced the *rcsA* promoter region by the phage lambda P_L promoter conferring constitutive *rcsA* expression (allele P_L*rcsA*). The *rcsA* promoter itself is H-NS repressed; its expression is positively autoregulated by RcsA-RcsB and requires induction of the Rcs signaling pathway (38). Constitutive expression of *rcsA* or stabilization of the RcsA protein in protease Lon mutants is sufficient for activation of RcsA-RcsB target genes in the absence of Rcs signal induction (37, 38). As expected, constitutive expression of *rcsA* (allele P_L*rcsA*) caused strong repression of the *flhDC* promoter-*lacZ* fusion (compare 228 units and 5 units corresponding to a 45-fold repression in Fig. 3). The mutations mapping in the overlapping YjjQ and RcsA-RcsB DNA-binding sites all reduced repression by RcsA-RcsB (Fig. 3). Interestingly, the G-to-T exchange at position +8 relative to the transcription start had the strongest effect and almost completely abrogated repression of the *flhDC* promoter by RcsA-RcsB, while the other mutations reduced repression by RcsA-RcsB from 45-fold to 7-fold. This +8G residue maps to the most conserved bases, GGA, of the RcsA-RcsB DNA-binding site (37). Taken together, the data show that YjjQ and RcsA-RcsB repress the *flhDC* promoter independently, that the YjjQ and RcsA-RcsB DNA-binding sites overlap, and that individual nucleotides are of different levels of relevance for repression by YjjQ and RcsA-RcsB.

Repression of motility by YjjQ and effects on biofilm formation. The promoter of the *flhDC* locus encoding the master regulator of flagellar synthesis is repressed by YjjQ, suggesting that YjjQ also represses motility of *E. coli*. To test this, the motile *E. coli* K-12 strain BW30270 was transformed with plasmid pKEKD31 carrying *yjjQ* under the control of the IPTG-inducible *tac* promoter as well as with the empty vector control. Motility assays using soft agar plates (0.25% agar) revealed a high motility of strain BW30270, which is presumably based on the insertion of an IS5 element upstream of the *flhDC* promoter (39). However, the motility was completely inhibited upon induction of *yjjQ* expression (Fig. 4A). Similar motility assays with UPEC strains 536 and CFT037 demonstrated that induction of YjjQ inhibits the motility of these strains as well (Fig. 4A). As regulation of flagellar synthesis may affect the motility-to-biofilm transition (40), we analyzed the effect of YjjQ on biofilm formation by the laboratory strain K-12 and UPEC strain CFT073 (Fig. 4B). Transformants of *E. coli* strains BW30270 (K-12) and CFT073 (UPEC) were grown in 96-well polystyrene microtiter plates for 48 h at 28°C, and biofilm formation was estimated by crystal violet staining (Fig. 4B). No staining was observed for UPEC strain 536 (data not shown), and therefore this strain was not used. In this assay, YjjQ inhibited biofilm formation by K-12 strain BW30270 but had no effect on UPEC strain CFT073 (Fig. 4B).

DISCUSSION

Previously, the presumptive transcriptional regulator YjjQ of *E. coli* has been implicated in virulence of the avian pathogenic *E. coli* strain IMT5155 (6), and *yjjQ* expression has been shown to be regulated by H-NS and LeuO (12). Here we have shown that YjjQ represses transcription of several loci by binding to a palindromic DNA-binding motif. These loci include the *flhDC* operon, encoding the master regulator of flagellar synthesis $FlhD_4C_2$, *gfc*, encoding enzymes of group 4 capsule (O-antigen) synthesis, *ompC*, encoding the outer membrane porin C, and *yfiRNB*, coding for a c-di-GMP cyclase and regulatory proteins, as well as two additional loci of poorly defined function. Furthermore, we show that YjjQ represses motility of the laboratory *E. coli* K-12 strain and of UPEC strains and that YjjQ inhibits biofilm formation by K-12 but not by UPEC strain CFT073. These data suggest that YjjQ represents a transcriptional repressor in *E. coli*, which may contribute to the switch between the motile and adhesive lifestyles.

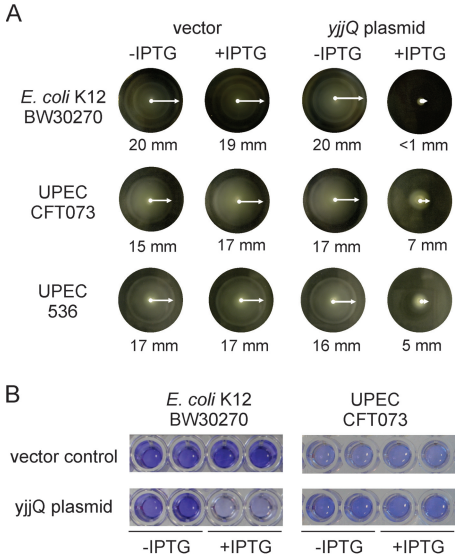

FIG 4 Control of motility and biofilm formation by YjjQ. (A) Soft agar plates (0.25% agar) were used to test the motility of transformants of K-12 strain BW30270 and UPEC strains CFT073 and 536 with the empty vector control and *yjjQ*-expressing plasmids pKEKD31 (for K-12) and pKEKD30 (for UPEC strains). The plates were incubated for 5 to 6 h at 37°C for strains K-12 and 536 and for 12 h at 28°C in the case of CFT073. The swimming radii are indicated. IPTG was added to a 200 μM final concentration where indicated. (B) Biofilm formation of transformants of K-12 strain BW30270 and UPEC strain CFT073 with the vector control and the *yjjQ* plasmids pKEKD31 and pKEKD30, respectively, was analyzed by crystal violet staining of bacteria adhering to polystyrol microtiter plates. Bacteria were grown for 48 h at 28°C before staining. IPTG was added to a 1 mM final concentration where indicated.

Transcriptional repression of the *flhDC* promoter by YjjQ adds an additional component to the complex regulation of this operon. In addition to the repression by YjjQ shown here, transcription initiation at the *flhDC* promoter is known to be controlled by OmpR, CRP, RcsA-RcsB and RcsB, HfdR, DksA/ppGpp, LrhA, and MatA (= EcpR) (37, 39, 41–46). Furthermore, several small regulatory RNAs (sRNAs) control expression of the *flhDC* operon at a posttranscriptional level (47). Tight control of *flhDC* expression, dependent on multiple inputs, is attributed both to the high energy consumption of flagellar synthesis as well as to the requirement to adapt a motile or sessile lifestyle, depending on the environment (48, 49). At the *flhDC* promoter, the YjjQ DNA-binding site and the RcsA-RcsB DNA-binding site overlap almost completely, but transcriptional regulation by YjjQ and that by RcsA-RcsB seem independent of each other. This may indicate that RcsA-RcsB and YjjQ compete for binding. However, another possibility is that repression by RcsA-RcsB or by YjjQ provides a means to control *flhDC* in response to different signals. RcsA-RcsB-mediated repression of *flhDC* is coupled to the Rcs signaling system that is sensing perturbation in the outer membrane protein assembly and peptidoglycan layer and is induced by antimicrobial peptides (50–53), while signals that induce *yjjQ* remain to be defined.

YjjQ also represses the *gfc* and *yfiRNB* operons, *ompC*, and two further loci (*ybhL* and *ymiA-yciX*). The *gfc*-encoded group 4 capsule is synthesized by various *E. coli* strains, as shown for enteropathogenic *E. coli* (EPEC) O127:H6, enterohemorrhagic *E. coli* (EHEC) O157:H7, and APEC O78:H9 (29, 54, 55). Furthermore, strains with mutation of the *gfc* locus in APEC O78:H9 strain X7122 are attenuated (54), while the group 4 capsule masks surface structures of EHEC O157:H7 and affects intestine colonization (55). The *gfc* locus is absent in UPEC strains (29). However, the *yfiRNB* operon, encoding a regulated c-di-GMP synthesis system, has been implicated in UPEC virulence, for which a *yfiR* mutant was shown to be attenuated in a murine urinary tract and kidney infection model (32). Thus, YjjQ represses a set of loci that are associated with several virulence traits in various pathogenic *E. coli* strains. A common feature of these loci is that they concern surface components (porin and capsule 4) and loci implicated in the control of the motile versus sessile lifestyle (*flhDC* and *yfiRNB*). However, a target gene relevant for iron homeostasis was not identified, although previous work suggested that YjjQ may control iron homeostasis in APEC (6). This discrepancy may be caused by a particular trait of the specific APEC IMT5155 strain or its derivative, the primary transposon-tagged *yjjQ* mutant that was analyzed previously. Such a trait might confer indirect control of iron metabolism by YjjQ.

How is YjjQ-mediated repression of *flhDC* and the other loci controlled? Expression of the *yjjQ* gene, which is encoded in the *yjjQ-bglJ* operon, is repressed by the global repressor H-NS and activated by the pleiotropic transcriptional regulator LeuO, as shown for the laboratory *E. coli* K-12 strain (12). LeuO, a regulator that is conserved in *Enterobacteriaceae*, is of pleiotropic function which includes virulence control in *Salmonella enterica*, and it is considered an antagonist of the global repressor H-NS (13, 15, 56). Present knowledge indicates that induction of the *yjjQ* gene determines whether repression by YjjQ occurs. It is not known whether YjjQ protein activity is also controlled. Furthermore, it needs to be worked out (i) how YjjQ integrates into regulatory networks controlling loci such as *flhDC*, *gfc*, *yfiRNB*, and *ompC* and (ii) how YjjQ may contribute to the control and modulation of virulence associated surface structures in various *E. coli* strains.

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